UNIVERSIDADE DE LISBOA
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Marine fish assemblages as indicators of anthropogenic pressures: identifying sensitive metrics

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Tese orientada pelo Professor Doutor Henrique Cabral e pela Professora Doutora Maria José Costa, especialmente elaborada para a obtenção do grau de Doutor em Biologia (especialidade de Biologia Marinha e Aquacultura)

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2013

“*It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.*”

Charles Darwin

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Abstract

Worldwide concern about the environmental threats and need for sustainable development has led to increased efforts to understand and assess anthropogenic pressure effects. However, the development of indicators for marine ecosystems is still at an early stage, due to their high spatial and temporal complexity. Based on several structural and functional traits (guild approach) and considering the effects of natural variability, the present study analysed the response of fish assemblages to several anthropogenic pressures in the Portuguese coast, by selecting fish-based metrics that best distinguish disturbed from control sites or those sensitive to gradients of pressure. In general, fish assemblages associated with both rocky reef and soft-substrate habitats were broadly affected by water pollution (sewage discharges and non-point sources of pollution), which led to changes in many metrics depending on the balance between the effluent toxicity and resources availability (e.g. trophic structure, resilience, habitat association and nursery function attributes). Conversely, fishing affected fish assemblages differentially, since in addition to the metrics related with commercial value, differences were only observed in tolerant-opportunistic and large individuals in rocky reefs, and species exhibiting vulnerable traits and dominance in soft-substrate habitats. Moreover, seasonal variability can influence the patterns of some fish-based metrics and their ability to detect pressures. The selection of the warm season after the spawning period (July-October) seems to be the more adequate to detect changes in rocky reef fish (cost-efficient). Further research is needed for soft-substrate habitats in order to select the most suitable sampling season. Finally, due to the difficulty to assess single-pressures on a wide-ranging environment, often characterized by multiple pressure contexts, an approach based on the previous selection of the expected pressure sources and applying a directional monitoring plan to analyze if the biological indicators detect changes, is strongly recommended (more cost-efficient).

Keywords: Functional and structural traits; marine fish assemblages; rocky reef habitats; soft-substrate habitats; anthropogenic pressures.

Resumo

A preocupação global com as ameaças ambientais e a necessidade de um desenvolvimento sustentável tem levado a um crescente esforço para compreender e avaliar os efeitos das pressões antropogénicas. No entanto, o desenvolvimento de indicadores nos ecossistemas marinhos encontra-se ainda numa fase inicial, devido à sua elevada complexidade espacial e temporal. O presente estudo analisou a resposta dos peixes a várias pressões antropogénicas na costa portuguesa, através da selecção de métricas estruturais e funcionais sensíveis a gradientes de pressão, ou que melhor distinguiram as zonas perturbadas das de controlo. Em geral, as associações de peixes tanto em recifes rochosos como em substratos móveis foram amplamente afectados por águas poluídas (descargas de esgoto e poluição difusa), que levaram a alterações em várias métricas dependendo do balanço entre a toxicidade do efluente e os recursos disponíveis para os peixes. Em contrapartida, os efeitos da pesca foram mais selectivos uma vez que, para além das alterações na métricas relacionadas com o elevado interesse comercial, apenas se observaram diferenças em indivíduos de maiores dimensões e tolerantes-oportunistas nos recifes rochosos, bem como nas espécies com características mais vulneráveis e dominância de espécies nos substratos arenosos. A variabilidade sazonal pode afectar os padrões de algumas métricas e a sua capacidade de detecção de impactos. Nos recifes rochosos, a selecção da estação quente, depois da época de reprodução (Julho-Outubro), parece ser mais adequada para a detecção de alterações nos peixes, enquanto para os substratos móveis são necessários estudos para definir a melhor época de amostragem. Devido à dificuldade de analisar pressões específicas num ambiente tão amplo e sujeito a pressões múltiplas, é recomendada a aplicação de uma abordagem baseada na identificação inicial das potenciais fontes de pressão e na aplicação de um plano de monitorização direccionado para por fim verificar se os indicadores biológicos detectam alterações.

Palavras-chave: Características funcionais e estruturais; grupos de peixes marinhos; recifes rochosos; habitats de substrato móvel; pressões antropogénicas.

Resumo alargado

Os ecossistemas marinhos contêm uma elevada complexidade de interacções bióticas e abióticas, responsáveis por uma série de processos ecológicos vitais à manutenção da própria vida. No entanto, muitos destes ecossistemas encontram-se ameaçados face à crescente degradação provocada pelas actividades humanas, sendo evidente a necessidade de recuperar e assegurar a sua utilização sustentável de modo a garantir o seu bom funcionamento. Neste sentido, é essencial compreender de que forma as actividades humanas afectam as comunidades biológicas e os limites de pressão que essas comunidades conseguem suportar sem que haja alterações no seu funcionamento.

Apesar de nas últimas décadas se terem desenvolvido vários indicadores para avaliar o estado dos ecossistemas aquáticos (e.g. estuários, rios), estes encontram-se ainda numa fase inicial no que diz respeito ao meio marinho, devido à sua grande complexidade espacial e temporal. Contudo, tem sido demostrado que a avaliação das alterações nas comunidades biológicas através de categorias estruturais e funcionais constitui uma abordagem eficiente, sensível e versátil, tendo levado a uma mudança de paradigma, em que as abordagens tradicionais (ao nível das espécies) têm sido gradualmente substituídas por abordagens baseadas em métricas estruturais e funcionais.

O presente estudo teve como principal objectivo avaliar a resposta estrutural e funcional de grupos de peixes associados a recifes rochosos e a habitats de substrato móvel a várias pressões antropogénicas, por forma a: identificar métricas sensíveis que possam ser utilizadas como indicadores; melhorar o conhecimento sobre as consequências dessas pressões antropogénicas; e contribuir para a correcta detecção das mesmas face à elevada dinâmica e dimensão dos ambientes marinhos. Esta tese é composta por sete capítulos, cinco dos quais referem-se a artigos científicos, publicados ou em revisão em revistas internacionais de arbitragem científica indexadas no *Science Citation Index*. Estes capítulos são precedidos por uma introdução geral e sucedidos por um capítulo de conclusões e comentários finais que incluem sugestões para estudos futuros.

No capítulo 1, introdução geral, é apresentado um enquadramento do tema da presente tese, onde são abordados os principais factores responsáveis pelos processos ecológicos e as ameaças provocadas pelas actividades antropogénicas. São também descritas as

principais dificuldades, a importância e os avanços relativos ao uso de peixes como indicadores de qualidade ambiental, assim como o seu enquadramento legislativo.

No capítulo 2 foi seleccionado um conjunto alargado de métricas relativas aos atributos de diversidade, abundância, estrutura trófica, mobilidade, resiliência, associação ao habitat e função de viveiro, que se pretende serem representativos das principais características das associações de peixes associados a recifes rochosos e das alterações esperadas face a pressões antropogénicas. Recorrendo a este conjunto de métricas, a resposta dos peixes foi analisada na presença de pressões da pesca, actividade portuária, descarga de esgoto e efluente térmico. Com excepção deste último, foram obtidas diferenças estruturais e funcionais significativas entre os locais perturbados pelas referidas pressões e os respectivos locais de controlo (com semelhante complexidade). Estas diferenças sugeriram a existência de dois padrões de resposta principais, consoante o número de atributos afectados: pressão selectiva, que afecta diferencialmente os grupos de peixes (pesca); e pressão abrangente, com métricas dos vários atributos analisados a responderem à sua presença (descargas de esgoto e actividades portuárias). Por fim, as métricas relativas a indivíduos generalistas, territoriais, de grandes dimensões com interesse comercial médio ou elevado, juvenis e ainda as métricas relacionadas com a estrutura trófica (excepto os zooplanctonívoros), foram seleccionadas como as mais sensíveis para avaliar alterações nos peixes de recifes rochosos. Estes resultados constituíram a base de referência para a selecção das métricas utilizadas subsequentemente no capítulo 3.

O efeito da sazonalidade nas métricas e a sua influência na detecção de um gradiente de pesca foi averiguado no capítulo 3. Apesar de ser expectável que as métricas sejam mais resilientes aos efeitos da variabilidade natural, em comparação com as espécies, algumas apresentaram variações ao longo das estações do ano analisadas, salientando-se as métricas relativas a juvenis, omnívoros e indivíduos que se alimentam de invertebrados. Os resultados revelaram diferenças claras entre as estações quentes (Verão e Outono) e frias (Inverno e Primavera), sugerindo que os padrões de variação encontrados se deveram aos processos de recrutamento, migrações de reprodução e ainda a movimentos de alimentação que ocorrem ao longo do ano. Para além disso, ficou demonstrado que estas variações sazonais podem causar dificuldades na detecção de pressões, uma vez que apenas foram encontradas diferenças nos indivíduos de elevado interesse comercial durante o Outono (métrica sensível ao efeito da pesca). Uma conclusão importante deste trabalho foi a selecção da época depois da reprodução para a maioria das espécies (Julho-Outubro), como a melhor altura para se avaliar alterações provocadas pelos

impactos antropogénicos em recifes rochosos. A selecção de uma época específica pode ter grandes implicações no melhoramento dos planos de gestão e na minimização dos custos das suas monitorizações.

Os capítulos 4 e 5 focam os efeitos de pressões antropogénicas sobre os grupos de peixes associados aos substratos móveis, através da análise dos gradientes de descargas de esgoto e de pesca com arrasto de fundo, respectivamente. Tal como no capítulo 2, foram utilizados conjuntos alargados de métricas, representativos da estrutura e função dos grupos de peixes característicos destes substratos e da sua resposta esperada perante as pressões mencionadas.

Desta forma, no capítulo 4 foi definido um gradiente de influência do efluente de um emissário submarino, com base na dispersão verificada em estudos anteriores, onde foram distinguidas três zonas com base na distância à saída do emissário. Da análise das diferenças encontradas entre essas zonas revelou que este efluente, sobretudo composto por matéria orgânica, provocou alterações tanto ao nível funcional como estrutural das associações de peixes, especialmente detectáveis junto à saída do emissário. Aparentemente, o padrão de resposta resultou não só dos potenciais níveis de toxicidade do efluente que levou ao decréscimo da abundância e biomassa de grupos de espécies mais sensíveis (resiliência baixa e muito baixa; Chondrichthyes), mas também do aumento de complexidade do habitat em consequência da presença das condutas. Essas condutas possivelmente funcionam como recifes artificiais, atraindo espécies tolerantes aos efeitos do efluente (residentes de rocha e omnívoros) que beneficiam dos novos recursos provenientes destes recifes (e.g. alimento, abrigo). Assim, pôde-se concluir que os efeitos das descargas de esgotos no meio marinho dependem dos aspectos estruturantes relativos à toxicidade do efluente e à complexidade do habitat, uma vez que estes condicionam a quantidade de recursos disponíveis para as espécies que tolerem os efeitos do efluente.

Por sua vez, no capítulo 5 foi utilizada uma abordagem inovadora de selecção de métricas que demonstrou ser extremamente útil na avaliação de zonas extensas que albergam um conjunto alargado de factores naturais (e.g. profundidade, latitude, substrato). Esta abordagem consistiu na modelação da resposta dos grupos de peixes a gradientes de intensidade de pesca com arrasto, que por sua vez foram definidos recorrendo às localizações transmitidas pelas embarcações via satélite (*Vessel Monitoring System* data) e analisadas com técnicas de Sistemas de Informação Geográfica (SIG). As métricas foram posteriormente seleccionadas de acordo com a sua consistência ao longo dos modelos de resposta nas quatro tipologias de habitats definidos *a priori*, ou seja, níveis de

intensidade de pesca com arrasto. No geral, as métricas relacionadas com indivíduos de níveis tróficos mais elevados, de elevado interesse comercial, que exibem características mais vulneráveis (Chondrichthyes, resiliência muito baixa, sedentários) e ainda a dominância revelaram-se mais sensíveis ao aumento da intensidade da pesca. Este padrão foi atribuído ao conjunto de possíveis efeitos directos e indirectos da pesca de arrasto que actuam sinergicamente sobre características específicas de associações de peixes associados a substratos móveis, levando à sua homogeneização.

Tendo em conta que os ecossistemas marinhos estão frequentemente sujeitos a impactos múltiplos provenientes de diferentes fontes de pressão, a detecção dos efeitos singulares de uma pressão específica é muitas vezes ocultada pelas diferentes pressões que actuam num mesmo local, constituindo, no entanto, um dos passos fundamentais para o sucesso dos planos de gestão. Neste contexto, a capacidade de detecção de pressões específicas foi analisada no capítulo 6, utilizando peixes e macroinvertebrados como indicadores biológicos e comparando a sua resposta. Para isso, foram definidos quatro tipos de gradientes de pressão (pesca, poluição orgânica, estruturas físicas e poluição difusa) com base na localização espacial e grau de impacto esperado das várias pressões existentes numa extensa área marinha (SIG). Estes gradientes serviram de base para modelar a resposta dos indicadores referidos não só às pressões específicas mas também ao padrão cumulativo dessas pressões. Ambos os indicadores foram concordantes na identificação dos locais sujeitos a maior pressão cumulativa, e a análise da resposta esperada das métricas sensíveis aos gradientes de pressão específica indicou que a contaminação difusa foi a pressão que mais contribuiu para os padrões encontrados. Uma vez que era expectável que outras fontes de pressão tivessem sido detectadas, foi sugerida uma nova abordagem para melhorar a avaliação de áreas extensas e sujeitas a pressões múltiplas, que consiste na identificação prévia das fontes de pressão que actuam numa determinada zona, juntamente com o delineamento de um plano de monitorização direccionado à origem dessas pressões, para que desta forma seja possível avaliar correctamente a resposta dos indicadores biológicos.

Finalmente, no capítulo 7 são apresentadas as várias conclusões que integram os principais resultados obtidos nos capítulos anteriores sendo também exploradas as implicações das abordagens utilizadas ou sugeridas no contexto da avaliação e detecção de pressões humanas nos ecossistemas marinhos. Neste capítulo são ainda propostas algumas linhas de investigação futura, que, de acordo com os resultados obtidos, irão complementar o conhecimento adquirido com o presente trabalho.

List of papers

This thesis is comprised by the papers listed below, each corresponding to a chapter, from 2 to 6. The author of this thesis is the first author in all papers and was responsible for conception and design of the work, field surveys, sample collection and processing, laboratory analytical procedures, data analysis and manuscript writing of all the papers. Remaining authors collaborated in some or several of these procedures. All papers published were included with the publishers' agreement.

CHAPTER 2: Response of fish-based metrics to anthropogenic pressures in temperate rocky reefs
Sofia Henriques, Miguel P. Pais, Marisa I. Batista, Maria J. Costa, Henrique N. Cabral
Published in Ecological Indicators (2013) 25: 65-76.

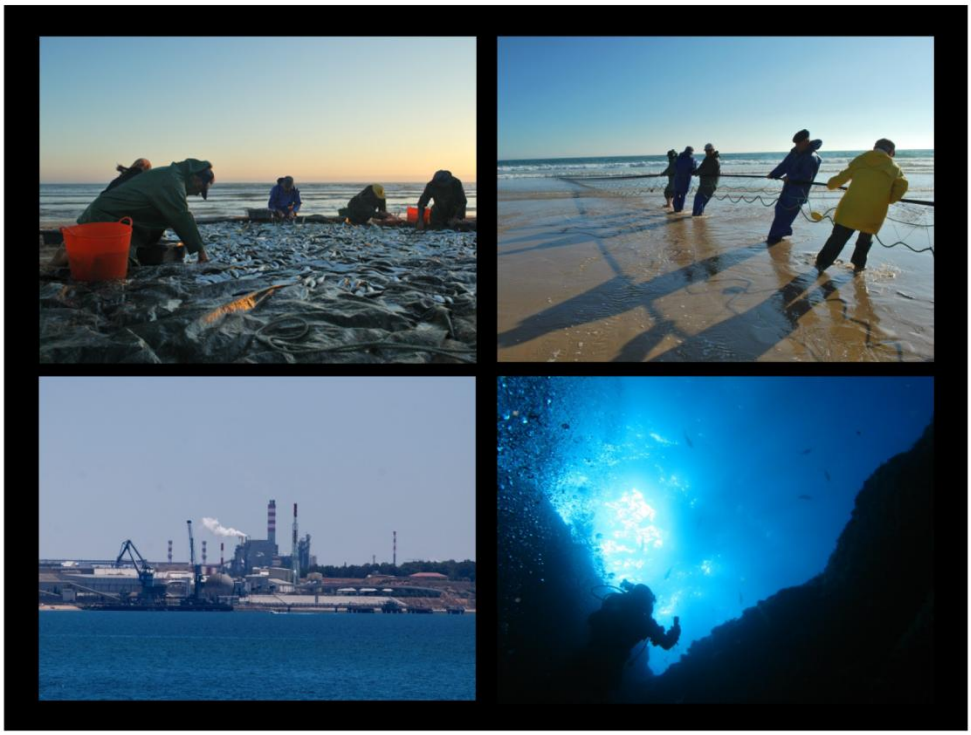
CHAPTER 3: Seasonal variability of rocky reef fish assemblages: detecting functional and structural changes due to fishing effects
Sofia Henriques, Miguel P. Pais, Maria J. Costa, Henrique N. Cabral
Published in Journal of Sea Research (2013) 79: 50-59.

CHAPTER 4: Structural and functional changes in a soft-substrate fish assemblage induced by submarine sewage outfall
Sofia Henriques, Miguel P. Pais, Maria J. Costa, Henrique N. Cabral
In review in Environmental Monitoring and Assessment.

CHAPTER 5: Structural and functional traits indicate fishing pressure on marine fish assemblages
Sofia Henriques, Miguel P. Pais, Rita P. Vasconcelos, Alberto Murta, Manuela Azevedo, Maria J. Costa, Henrique N. Cabral
In review in Journal of Applied Ecology.

CHAPTER 6: Can different biological indicators detect similar trends of marine ecosystem degradation?
Sofia Henriques, Miguel P. Pais, Marisa I. Batista, Célia M. Teixeira, Maria J. Costa, Henrique N. Cabral
Submitted to Ecological Indicators.

CHAPTER 1



General introduction

Marine ecosystems: ecological processes and threats

Fish assemblages as indicators of anthropogenic pressures

General aims and thesis outline

General Introduction

Marine ecosystems: ecological processes and threats

Marine environments comprise complex networks of interactions among the assemblages of living organisms (biotic component) and between those organisms and the abiotic environment (physical and chemical components) (Costanza & Mageau 1999; Cury et al. 2003; Mann & Lazier 2006; Costello 2009). Besides providing food and raw material (i.e. goods), these multiple interactions result in several ecosystem processes that are essential to the proper functioning of the Earth (i.e. services), such as the regulation of climate, the bioremediation of pollutants and waste, the prevention of flood and storm, the buffering of climate change and regulation of nutrient cycling (Costanza & Mageau 1999; Beaumont et al. 2007; Bremner 2008). However, marine ecosystems are subject to a wide range of threats that often lead to their degradation with consequent decline or loss of those functions (Hooper et al. 2005; Bremner 2008; Mouillot et al. 2012). Therefore, understanding how anthropogenic impacts affect the complexity of the physical, chemical and biological interactions, as well as the limits of pressure intensity between which biological assemblages can stand without causing a shift to an alternative state, is becoming a crucial challenge in order to ensure sustainability of those assemblages (Costanza & Mageau 1999; Cury et al. 2003; Hughes et al. 2005; Borja et al. 2012).

The concept of sustainable ecosystem, *sensu* healthy ecosystem, is directly related with ecosystem's capacity of maintaining its structure and function (integrity) over time in face of external stress (resilience), that in turn is supported by synergetic feedbacks between the biotic and abiotic components (Figure 1.1) (Costanza & Mageau 1999). These biotic (e.g. tolerance, adaptation, recruitment, competition) and abiotic factors (e.g. habitat complexity, temperature, wind, currents) determine the spatial and temporal homogeneity of marine assemblages (Rice 2005; Johnson et al. 2012). The diversity and distribution of marine assemblages depends on the species life-cycles and their connections with the surrounding habitat, through the balance among the ecological needs, resources availability (e.g. food, shelter, conditions that maximize the recruitment), physiological tolerance and capacity of adaptation (Figure 1.1) (García-Charton & Pérez-Ruzafa 2001; Pihl & Wennhage 2002; Rice 2005). In addition, it is well known that the patterns of marine

biological assemblages vary at different temporal scales (i.e. seasonal and inter-annual variability) due to natural environmental oscillations (e.g. sea temperature, currents, upwelling events) that trigger processes such as species migration, spawning seasons and recruitment (Holbrook et al. 1994; Harmelin-Vivien et al. 1995; Friedlander & Parrish 1998; Aburto-Oropeza & Balart 2001; Henriques et al. 2007). For instance, in the Portuguese coast, the variation in winter sea conditions associated with the North Atlantic Oscillation (NAO) is possibly the main factor responsible for the inter-annual variation in a marine fish assemblage by causing shifts in sea surface temperature (SST), currents and wind direction (see Henriques et al. 2007 for details). These oceanographic conditions can cause changes in the proportion of some species by affecting the transport of eggs, larvae and juveniles from other biogeographic regions, as well as affect the local recruitment patterns due to changes in wind direction (e.g. offshore transport or larval retention) (Henriques et al. 2007). Seasonality affects the arrangement of fish assemblages as a consequence of differential patterns in the distribution of some species, for example: appearance of juveniles and reproductive fish at a particular habitat and/or depth range and season and occurrence of planktonivore species associated with upwelling events (Gaertner et al. 1998; Sousa et al. 2005).

Furthermore, the degree to which ecosystems and assemblages are affected by environmental and anthropogenic disturbances is related with the complexity of trophic relationships through the dynamic processes of the bottom-up, wasp-waist and top-down control (Caddy & Garibaldi 2000; Pennigar et al. 2000; Cury et al. 2003). In its simplest form, top-down control is the process where variations in the upper levels of food web, usually top-carnivores, drive the abundance of the lower levels, each trophic level influencing the one below. In bottom-up control the process starts from lower trophic levels and continues upwards through the food web. Wasp-waist process occurs when an intermediate level of the food web (e.g. planktonivores), which depends on the environment (e.g. upwelling), affect the abundances of the upper and lower levels. In this context and since species and assemblages do not exist in isolation, both environmental and anthropogenic pressures are likely to affect the overall productivity of the ecosystems (Figure 1.1) (Cury et al. 2003).

Over-harvesting, pollution and the impacts of climate change as a result of several anthropogenic activities have been largely recognized as the primary threats on marine ecosystems (Islam & Tanaka 2004; Hughes et al. 2005; Crain et al. 2009), causing dramatic shifts in marine assemblages composition and consequently unstable systems (Graham & Harrod 2009; McKinley & Johnston 2010; Johnson et al. 2012).

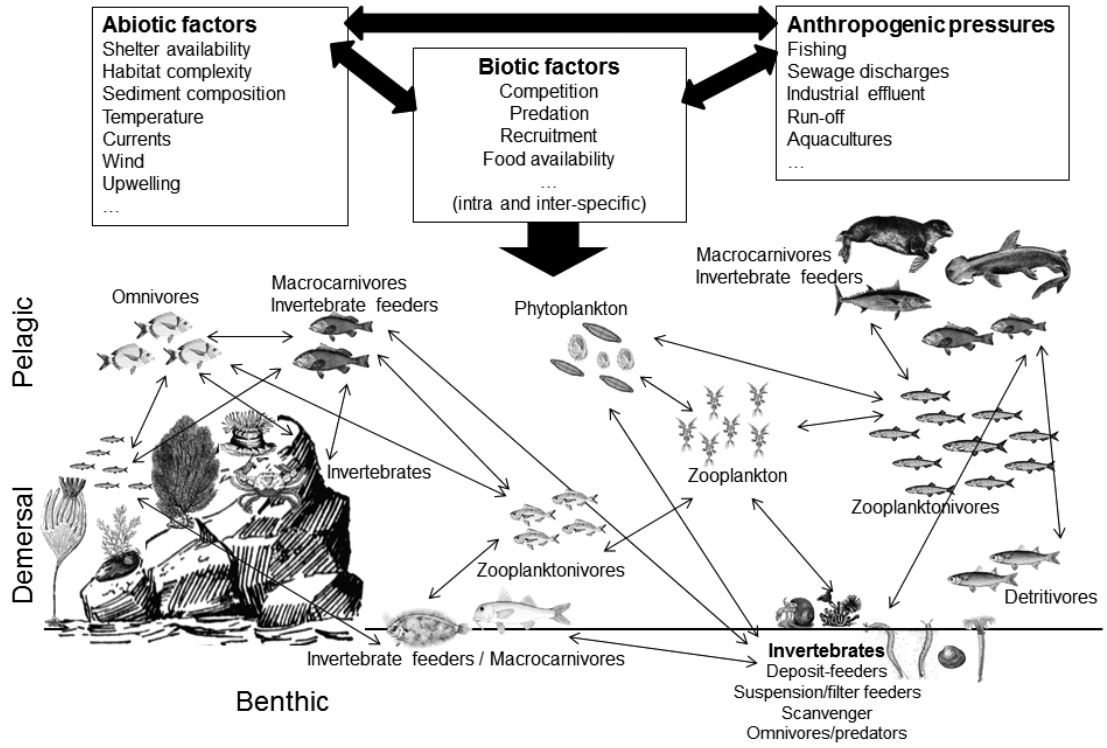


Figure 1.1 Illustration of the complexity of interactions among anthropogenic pressures, environmental and biotic factors on a simplified food web. Arrows represent the direction of those interactions. Images are from Clipart courtesy FCIT (http://etc.usf.edu/clipart/).

In fact, over-harvesting, mostly associated with fishing activities, can lead to the reduction of living resources (both target and non-target species) and to the destruction of their habitats with likely profound effects on food webs that ultimately change the structure and function of ecosystems (Caddy & Garibaldi 2000; Cury et al. 2003; Crain et al. 2009). Pollutants derived from a variety of sources, such as domestic and municipal wastes (organic compounds, pathogens, heavy metals and trace elements), agriculture (fertilizers, pesticides and agrochemicals), aquaculture (alien species, sediments and organic compounds), industrial activities (heavy metals and trace elements), shipping (oil spills, invasive species, noise), among others. These pollutants can cause direct and indirect effects on marine organisms by affecting their survival, growth, reproductive success, food availability and interfering on metabolic processes, while increasing their susceptibility to diseases and deformities, depending on the pollutants toxicity and concentrations (see Islam & Tanaka 2004; Crain et al. 2009; McKinley & Johnston 2010). Warning temperatures of ocean and air, increasing rates of sea-level rise, ocean acidification and UV exposure are some of the major impacts of climate change, which affect the metabolic

processes and the biogeographical distribution of species with consequences in the diversity, structure and function of marine assemblages, in addition to the deep implications in the loss of important ecosystems (e.g. polar areas, coral reefs, coastal habitats) (Crain et al. 2009). Overall, these impacts tend to be intensified due to the increases in industrialization, population growth and current levels of ecosystems degradation, which stress the need of ranking the ecosystems vulnerability and key threats in order to prioritize conservation efforts and direct management measures to reduce those impacts (Crain et al. 2009; Ban et al. 2010).

It has been estimated that a third of the world´s oceans are under medium to very high cumulative impact levels, but these levels are unequally distributed, mostly concentrated in the continental shelf and slope, as a result of both land- and ocean-based anthropogenic pressures, while the less impacted areas occur in the poles (Halpern et al. 2008). Moreover, anthropogenic activities with significant impacts (fishing, aquaculture, coastal engineering and pollution) primarily affect the intertidal and nearshore ecosystems with coral reefs, rocky reefs and mangroves pointed out as the most threatened marine ecosystems, as well as hard-bottom shelf areas (30-200m) (Halpern et al. 2007; Halpern et al. 2008; McKinley & Johnston 2010). Conversely, shallow soft-bottom and pelagic deep-water ecosystems are the less threatened due to their lower vulnerability (Halpern et al. 2007; Halpern et al. 2008). Despite this worrying global overview, the effects of these impacts on marine biological assemblages and their consequences for the ecosystem remain poorly understood.

Until recently, investigations about the anthropogenic impacts on biological organisms focused on taxonomic-based approaches, by employing species richness, diversity indices, evenness or population abundance as descriptors (Niemi & McDonald 2004; Mouillot et al. 2012). Theoretical ecological foundations suggest that, under stable conditions, marine assemblages are characterized by a strong interspecific competition resulting in a balance between large-body size, slow growth, long life span species (supposedly more sensitive) and opportunistic species (short life span, fast growth), with those sensitive dominating the assemblages in terms of biomass. Conversely, under increases of stress, those assemblages become gradually shifted to opportunistic species which, at highest levels of disturbance intensity, dominate the assemblages in both abundance and biomass (Warwick 1993; Yemane et al. 2005; Cheung et al. 2008). However, since not all species are ecologically identical and in view of the above-mentioned biotic and abiotic interactions, the complexity of human-induced changes cannot be solely viewed as species differences in terms of tolerance to disturbance as the

stress-response relationships are far from be unimodal (Hughes et al. 2005; Mouillot et al. 2012). Consequently, the approaches are gradually becoming more ecological, moving from the assessment of individual species at a single broad scale, to multiple-species analyses, and finally to ecosystem-based assessments at multiple spatial and temporal scales (Levin & Lubchenco 2008).

In the last decades the ecosystem-based approach (EBA) has become a new and central paradigm underlying international policies, such as the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD), which consider the entire ecosystem components (including humans), their interactions and the impacts of multiple activities, as integral parts of the marine management in order to ensure sustainable ecosystems. For instance, the MSFD aims to achieve good environmental status of all EU marine waters by 2020 and to implement programmes of measures in order to recover impacted systems and prevent future degradation (Directive 2008/56/CE). The implementation of the MSFD should include an integrated approach to assess the environmental status, comprising several biological elements such as plankton, algae, macroinvertebrates, fish, marine mammals, reptiles and seabirds, together with physical and chemical features (see annex III in Directive 2008/56/CE). Since the previous assessment tools focused on physicochemical targets, these legislative requirements bring new challenges and urgent demand of the: development of biological tools and methodologies to accurately assess to the environmental status of marine waters and establish the ecological quality objectives; as well as the application of an approach based on an adaptive management to deal with political and environmental changes.

Fish assemblages as indicators of anthropogenic pressures

The extreme difficulty of measuring anthropogenic impacts in such complex, spatially and temporally diverse ecosystems led to the development of several indicators in order to isolate key aspects that provide insight into changing conditions (Heink & Kowarik 2010). In the ecological and environmental planning contexts the meaning of the term indicators is still ambiguous as it varies widely in usage, often with different terms used as synonyms (see Heink & Kowarik 2010 for further details). Since indicators are relevant to link science and policy, presenting a definition in the context in which they are applied becomes important to avoid misinterpretations (Heink & Kowarik 2010). Here, the meaning inherent to environmental indicators implies that measures of biological, physical or chemical components reflect changes in the environment state (evaluative indicators), while ecological indicators are measurable characteristics of biological organisms (from cell to

community level) used to assess the condition of the ecosystem (state of ecological processes) and to detect change related to anthropogenic disturbances (descriptive indicators). These concepts were defined based on the revisions of Niemi et al. (2004), Niemi and McDonald (2004) and Heink and Kowarik (2010).

Independently of the definition used, all indicators must detect and quantitatively assess anthropogenic impacts against a background of natural variability in a predictable manner, be early-warning signals of disturbance, be cost effective as well as have a broad applicability (different geographical areas and habitat types) (Dale & Beyeler 2001; Greenstreet & Rogers 2006). In this context, the assessment of changes through functional and structural guilds (i.e. metrics based on biological traits), has proven to be a versatile, powerful and sensitive approach, since species within guilds tend to be similarly affected by impacts and, along spatial and temporal gradients, they are replaced by others sharing the same guild (Micheli & Halpern 2005; Elliott et al. 2007; Noble et al. 2007; Bremner 2008; Mouillot et al. 2012; Pais et al. 2012).

In the present study, fish assemblages will be the ecological indicator analysed. Although some limitations have been identified in their use as indicators, such as the selectivity and seasonal nature of samples, the large sampling effort that may be required to be representative, their mobility and relative tolerance to chemical pollution, these limitations are largely offset by the advantages: trophic position (high variety of trophic levels, including those near the top of food webs), easiness in identification when compared with other biological groups, extensive life-history information available, high variety of functional guilds that reflect several components of the ecosystem; In addition, fishes are both sedentary and mobile (given us information about the local and “border” of the effects), can show external anatomical pathologies, are relatively long-lived species providing temporal integration in the assessments and have high economic value making simpler to communicate with the general public (Harris 1995; Whitfield & Elliott 2002; Harrison & Whitfield 2006). In fact, previous studies showed that fish assemblages provide powerful tools for assessing streams and estuaries, i.e. multimetric indices – tools used to classify the condition of an environment according to the anthropogenic-induced changes in features of biological assemblages (e.g. Karr 1981; Deegan et al. 1997; Breine et al. 2006; Harrison & Whitfield 2006; Hering et al. 2006; Coates et al. 2007; Roset et al. 2007; Marzin et al. 2012). However, to our knowledge, the Marine Fish Community Index (MFCI) was the only tool specifically developed to assess marine fish assemblages (Henriques *et al.* 2008). Yet, the dataset used to perform the MFCI tests resulted from scientific reports and papers collection, and lacked information about the anthropogenic impacts affecting

the areas (Henriques et al. 2008). Therefore, the use of fish-based indicators in marine waters is still in an early stage, an there is an urgent need for scientific knowledge about the sensitivity and consistency of metrics (i.e. measures that describe features of the structure and function of marine biological assemblages) in the assessment of anthropogenic pressures.

General aims and thesis outline

In view of the growing awareness of assessing human-induced changes at ecosystem level, including the legislative requirements, as well as the lack of information available for the use of fish assemblages as indicators in marine waters, the general aims of this thesis were: (1) to identify sensitive metrics, based on marine fish assemblages associated to different habitats and their responses to the main anthropogenic pressures (2) to improve the current understanding about the consequences of anthropogenic pressures in fish assemblages, (3) to critically address the precautions needed to properly detect disturbed areas on wide-ranging and dynamic environment. The thesis includes five scientific papers published, in review or submitted in peer reviewed international journals, each corresponding to a chapter.

In all chapters, the approaches applied were designed to focus on changes caused by anthropogenic pressures while considering the effects of natural variability. Therefore, disturbed areas were compared with control areas or along pressure gradients whilst accounting for season and the similarity of environmental features (e.g. rocky reef complexity, depth, sediment type). Given that habitat complexity plays an important role in the spatial and temporal composition of fish assemblages, due to the above-explained biotic and abiotic interactions, fish assemblages associated with soft-substrates and rocky reefs were analysed separately.

Chapter 2 relies on both structural and functional responses of rocky fish assemblages to the pressures of fishing, sewage discharges, port activities and thermal effluents in order to select trait-based metrics that best distinguishes disturbed from control areas. One of the novel aspects is the integrated assessment achieved through the analysis of several metrics representing numerous attributes of fish assemblages (namely diversity, abundance, trophic structure, mobility, resilience, habitat association, nursery function). The results are discussed in the light of studies focusing on anthropogenic impacts in order to identify the possible mechanisms driving the observed patterns. This chapter identifies a set of sensitive metrics with biological meaning that will be the basis of the next chapter.

Seasonal variability is one of the main drivers of fish distribution and abundance variations in rocky reefs. However, only a few studies analyzed the effects of seasonal variability on marine rocky fish assemblages and none focused on fish metrics, which is key to improve the understanding of anthropogenic impacts. Chapter 3 addresses the effects of seasonal variation on the stability of several trait-based metrics (guild approach) as well as their capability to detect the effects of fishing pressure. The results are discussed regarding the choice of the best season to assess anthropogenic pressures.

Chapters 4 and 5 address the use of soft-substrate fish assemblages in the assessment of human-induced changes. Chapter 4 constitutes the first guild approach to the effects of sewage on both structural and functional fish-based metrics. Chapter 5 examines changes in fish assemblages structure and functioning concurrent with changings levels of trawling effort, one of the most destructive fishing methods. Moreover, in chapter 5 a novel approach is applied by comparing the response models of several trait-based metrics and the consistency of metric response among different soft-substrate habitat typologies. Results of both chapters are discussed in relation to the possible factors responsible for the observed changes and indicate a set of sensitive metrics that will be used in chapter 6.

Since marine ecosystems are usually under the influence of multiple-pressure sources, that can mask the response of indicators, chapter 6 tests if known sensitive trait-based metrics of both fish and macroinvertebrate indicators are capable of detecting ecosystem degradation problems, and distinguishing pressure types (i.e. fishing, organic, physical and non-point source). The results are discussed regarding the design of more cost-efficient field surveys.

Finally, chapter 7 outlines the main conclusions gathered from the several chapters, their contribution and implications within the context of anthropogenic impact assessment. This chapter also point out some recommendations on how adequately assess disturbed areas on wide-ranging and dynamic ecosystem and presents suggestions for further research.

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CHAPTER 2

Henriques S., Pais M.P., Batista M.I., Costa M.J. & Cabral H.N. (2013). Response of fish-based metrics to anthropogenic pressures in temperate rocky reefs. Ecological Indicators 25, 65-76.

Response of fish-based metrics to anthropogenic pressures in temperate rocky reefs

Abstract: The increasing degradation of marine ecosystems as a result of increasing impact caused by anthropogenic pressures, urges for well-founded knowledge to develop efficient tools to appraise the quality status of fish assemblages, as required by the Marine Strategy Framework Directive. This study analyzed the structural and functional response of rocky fish assemblages to several pressures on the Portuguese coast, i.e. fishing, sewage discharges, port activities and thermal effluent, by selecting fish-based metrics that best distinguished disturbed from control areas. One of the novel aspects of this research is the integrated assessment made through the analysis of several metrics representing numerous attributes of fish assemblages (namely diversity, abundance, trophic structure, mobility, resilience, habitat association, nursery function), which contrasts with the most commonly used approaches that in general focus on fish species/families. PERMANOVA results showed significant differences on metrics composition for all pressures with the exception of the thermal effluent. Moreover, two major patterns of stress were identified: (1) selective pressure, which affects differentially the fish assemblages (fishing); and (2) broad-range pressure, which affects the entire fish assemblage with metrics of several attributes (e.g. structure, resilience, trophic guilds, nursery function) responding to its presence (sewage discharges, port activities). Taking into account the sensitivity results (discriminant analysis and Mann-Whitney test), biological meaning and redundancy with other metrics (Spearman correlations), the following metrics were selected as the most suitable to detect changes on temperate reef fish assemblages: density of generalist individuals, density of territorial individuals, density of large individuals with medium to high commercial value (> 20 cm), density of juveniles and metrics relative to trophic guild (except zooplanktivores). Since metrics grouped species that have some degree of functional overlap, the present approach was useful to understand human-induced changes at the assemblage level, contributing for the future use of marine fishes as biological indicators.

Keywords: Fish-based metrics; multi-stressor approach; temperate rocky reefs; environmental quality assessment, Portugal, Marine Strategy Framework Directive (MSFD).

Introduction

Marine ecosystems are influenced by several land- and ocean-based human activities that are responsible for their degradation, which is being intensified with human population growth, especially on coastlines (Halpern et al. 2008; Crain et al. 2009). Consequently, quality assessment and monitoring of marine ecosystems has become increasingly important to ensure their sustainability (e.g. Directive 2008/56/CE ; Spatharis & Tsirtsis 2010; Borja et al. 2012). There is widespread agreement that water pollution provided from variable sources (e.g. agriculture, aquaculture, industrial and urban wastes), fishing, dredging, port activities, coastal engineering and biological pollution are amongst the major

threats of the marine ecosystems health (Halpern et al. 2008; Crain et al. 2009; Ban et al. 2010). In this context, knowing the location and effects of anthropogenic activities on marine communities is critical to successful management and conservation (Ban et al. 2010; Korpinen et al. 2012).

Several studies have shown that marine fish assemblages associated with hard substrates respond to human-induced changes (e.g. Khalaf & Kochzius 2002; Guidetti et al. 2003; Claudet et al. 2006; García-Charton et al. 2008; Azzurro et al. 2010; McKinley & Johnston 2010). However, the differences found between disturbed and control areas focused on fish species/genus/families with few metrics related to functional guild composition employed, and even when used, they tend to represent only one feature (e.g. trophic structure). Since anthropogenic activities can have a direct influence on food resources, distribution, diversity, breeding, abundance, growth and survival of fish assemblages (Henriques et al. 2008 and references therein), the usual approach is insufficient to characterize both functional and structural changes of the whole assemblage. Therefore, an integrative analysis of pressure-response relationship of several fish-based metrics by comparing different pressures is required (multistressor approach), towards the development of efficient tools to assess the quality state of marine fish assemblages (e.g. multimetric indices).

The complexity of rocky reefs plays a key role in determining the diversity and spatial distribution patterns of fish assemblages depending on species life-cycles, by limiting the quantity of food and shelter available, density of predators and quality of nursery habitats (Rice 2005). Therefore, the study of the potential fish assemblages a given habitat can support is extremely important in order to successfully understand the effects of anthropogenic impacts (García-Charton & Pérez-Ruzafa 2001).

Based on habitat characteristics, disturbed and their respective control sites were selected on the Portuguese coast in order to represent the impacts of fishing, sewage discharges, port activities and thermal effluents. By using underwater visual census (UVC) along strip transects, fish assemblages associated with each site were characterized through several fish-based metrics, representing both functional and structural features of the assemblages. Finally, the effects of the presence of the above-mentioned pressures on fish assemblages were tested through: (1) the selection of the fish-based metrics that best distinguish disturbed from control sites and (2) the characterization of the stressor-response patterns of fish-based metrics.

Material and Methods

Study areas

A total of eight sites on the Portuguese west coast were selected, based on the presence of fishing activity, sewage discharges, port activities and thermal effluents. For each type of pressure a disturbed and a control site were sampled (henceforward designated as D and C, respectively) (Figure 2.1).

In the case of fishing, the disturbed site was located on the complementary protection zone in the Marine Protected Area of Arrábida (MPA), where local fishing activities with traps, nets, angling, longlines and handlines are allowed for licensed boats (< 7 m stern to bow), while its no-take zone (without any activity) was selected as control site (Figure 2.1a). The site disturbed by sewage discharges was located in Cape of Sines (Figure 2.1b), between a sewer and a runoff effluent of a stream that crosses the industrial zone of Sines, both discharging untreated waters directly to the sea close to the shoreline. The control site was located to the north, due to prevailing winds and oceanic swell from the northwest (Fiúza et al. 1982).

Concerning the pressure of port activities and accounting that the structure of their waterbreaks works as an artificial substrate (not comparable with natural rocky reefs), two sites inside the Sines harbour were selected. The disturbed site was placed inside the marina in the innermost area of Sines harbour, with the influence of several human activities including a large fishing harbour. The control site was located near a small yacht club with few small boats and human presence (Figure 2.1c). Regarding the thermal effluent, the disturbed site was located near the hot water output of the thermal power station of Sines and the control site located to the south, far from the influence of the hot water discharge, according to temperatures measured with a multiparameter probe YSI Professional Plus (Figure 2.1d). It is important to highlight that control sites do not represent “pristine” conditions, but since each analysed pressure prevails in the correspondent disturbed site, one can infer to a certain degree that differences among fish assemblages are probably due to the presence of the analysed pressure.

In order to ensure that each pair of sites (D vs. C) had comparable habitat complexity, they were characterized by the following measures: a 25 m chain positioned to follow the contours and crevices as closely as possible and the linear distance from the beginning to the end of the chain was used to estimate the rugosity ratio (Ferreira et al. 2001); Proportion of rock and sand cover (in meters) estimated along each deployment of the chain; algal cover, characterized by the mean percentage of cover (50 x 50 cm quadrat)

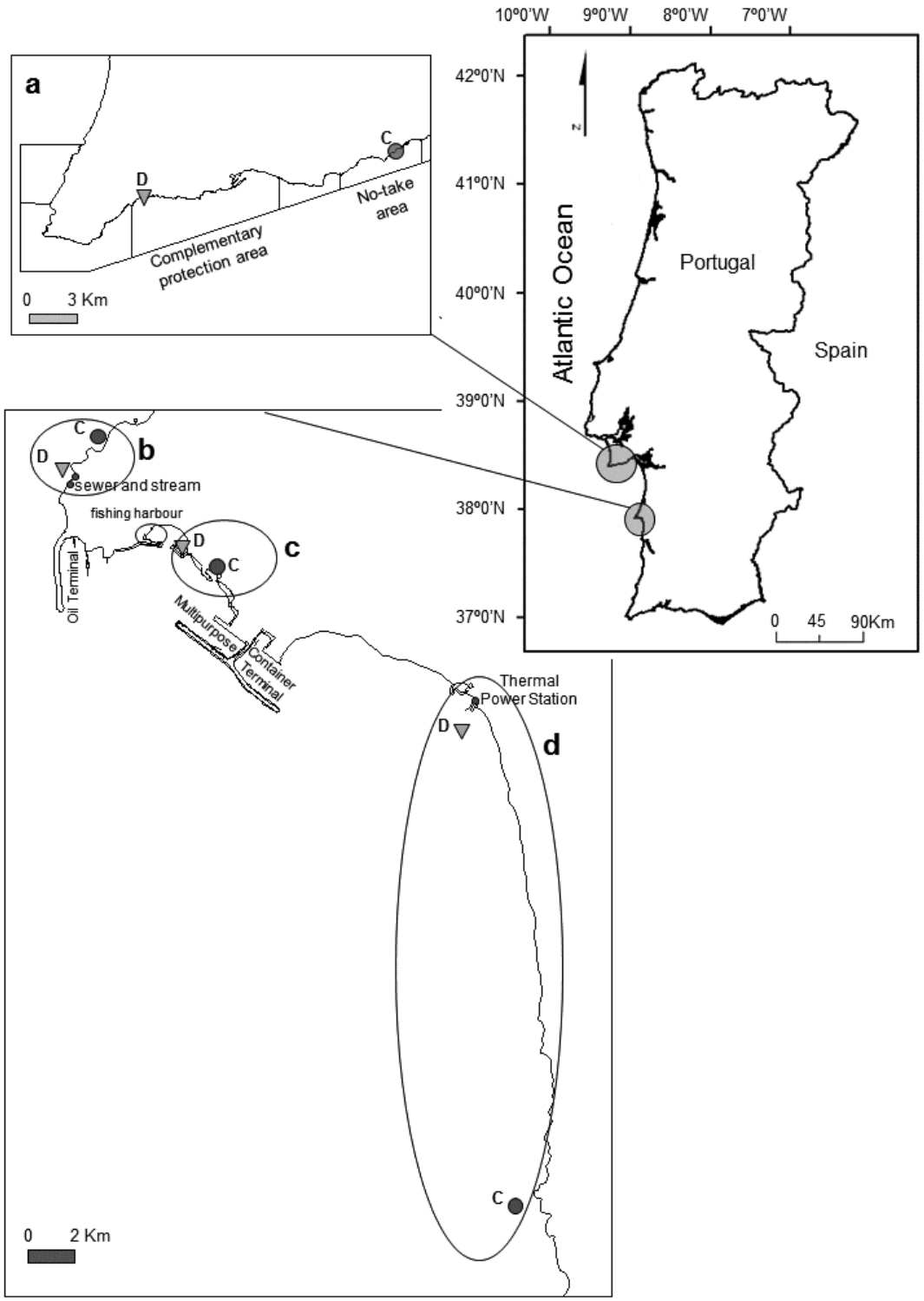

Figure 2.1 Location of the sampled sites that represent the pressures of: a - fishing; b - sewage discharge; c - port activities; and d - thermal effluent. Circles indicate the control sites (C) and triangles the disturbed sites (D).

by structural groups: encrusting, creeping (< 5 cm), tufts, filamentous and sheet (≥ 5 cm); invertebrates, the presence of sponges, anemones, hydrozoans, gorgonians, polychaetes, gastropods, crustaceans, sea urchins, starfish, sea cucumbers and ascidians was recorded in each quadrat (1 m^2). Habitat sampling was performed by depth strata (similar to the sampling of fishes, see below). Two chain deployments and six quadrats were performed in each stratum.

Fish sampling method

Fish were sampled using underwater visual census (UVC) through 50 m long strip transects placed parallel to the coastline. In order to direct the diver´s attention to both holes and crevices and water column, maximizing their representativeness, each transect was travelled twice for each replicate, first pass for non-cryptobenthic species (50 m x 2 m) and the second for cryptobenthic species (50 m x 1 m). These transects were performed with a minimum visibility of 5 m. In all transects the abundance and total length of observed species were recorded by the same divers (S. Henriques & M.P. Pais) in order to minimize observer effects.

A preliminary study using a total of 26 fish transects was performed, 13 allocated randomly in each depth strata (shallow 0-5 m; deep 5-10 m), sampled during the summer in consecutive days, at an independent site with high habitat complexity, to explore the number of replicates and divide the species between both transect passes, according to their behaviour. Length estimates were tested and calibrated between both observers until no significant differences were found. A total of 6 replicates per depth strata were assumed as representative of the fish assemblages. The species belonging to the families Blenniidae, Bothidae, Batrachoididae, Callionymidae, Congridae, Gadidae (subfamilies Phycinae and Lotinae), Gobiesocidae, Gobiidae, Muraenidae, Scorpaenidae, Scophthalmidae, Soleidae, Syngnathidae, Tripterygiidae, the species *Ctenolabrus rupestris* and *Labrus mixtus* as well as *Symphodus* spp. (with less than 5 cm total length) were counted on cryptobenthic transects.

A total of 60 transects were performed between spring 2010 and summer 2011 corresponding to 9000 m^2 of sampled area. Sites representing the fishing pressure included both depth strata, for port activities only the shallow stratum was present, while the remaining sites only have the deep stratum. Since season, sampling method, habitat and depth strata were similar between each D vs. C pair, fish assemblages were comparable per type of pressure.

Fish-based metrics

A list of candidate metrics was compiled from an extensive review of existing studies about fish response to anthropogenic pressures and description of rocky fish assemblages (Table 2.1) (Fasola et al. 1997; Mosqueira et al. 2000; García-Charton & Pérez-Ruzafa 2001; Guidetti et al. 2002; Khalaf & Kochzius 2002; Pelletier et al. 2005; Rice 2005; Claudet et al. 2006; Clynick 2006; Henriques et al. 2007; García-Charton et al. 2008; Harmelin-Vivien et al. 2008; Henriques et al. 2008; Pizzolon et al. 2008; Johnston & Roberts 2009; Azzurro et al. 2010; Claudet et al. 2010; McKinley & Johnston 2010; Wen et al. 2010). These metrics represent a range of structural and functional fish assemblage characteristics including diversity, composition, abundance, trophic structure, habitat association, nursery function, mobility and resilience. To test the thermal effluent pressure, metrics related with biogeographic affinities were added to the analysis (Table 2.1).

Density/abundance data are more sensitive to subtle changes in assemblages than relative frequencies or number of species (Hewitt et al. 2005; McKinley & Johnston 2010) and functional guilds tend to suffer smaller natural variations and respond more predictably to stress (Elliott et al. 2007). Thus, a guild approach was adopted and fish-based metrics were measured in density (ind. m^{-2}) (Table 2.1). Finally, all fish species were allocated to their ecological and functional guilds based on the previous classification of Henriques et al. (2008) updated with available literature and FishBase online database information (Froese & Pauly 2012) (Supplementary data I).

Metrics selection

Differences among the fish metrics of each pair of sites (D vs. C) were examined through one-way multivariate analysis of variance using permutations (PERMANOVA; Anderson 2001). This method does not assume normality since the p-values are obtained by permutations, but it is sensitive to differences in dispersion among groups, so homogeneity of multivariate dispersions was tested using the PERMDISP routine (Anderson et al. 2008). In order to understand the response patterns in multivariate space, unconstrained Principal Coordinates Analysis was used (PCO; Anderson et al. 2008). Moreover, to minimize the potential influence of microhabitat, PERMANOVA analyses were repeated excluding the cryptobenthic species belonging to the families Gobiidae, Bleniidae, Gobiesocidade and Tripterygiidae, since they depend directly on substratum type (Fasola et al. 1997). Despite this, a careful analysis was made considering the expected response of fish species and habitat features, to avoid misinterpretations (see discussion).

Table 2.1 List of candidate metrics to characterize the fish assemblage response to anthropogenic pressures. Metrics are divided by the following attributes: diversity/structure, trophic structure, mobility, resilience, habitat association, nursery function and biogeographic affinity.

Metric	Description
Species diversity/composition/abundance	
Total number of species	Measure of species richness
Total density	Measure of abundance
Density of individuals of rare and uncommon species	Measure of conservation value of the system
Dominance	Number of species that make up 90% of the total density
Density of individuals with medium commercial value	
Density of individuals with high commercial value	
Density of large individuals with medium to high commercial value (> 20 cm)	Metrics that have been used to measure the effects of fishing
Commercial/non-commecial ratio (in density)	
Trophic structure	
Density of invertebrate feeders	Feed predominantly on non-planktonic invertebrates
Density of omnivorous	Feed on detritus, filamentous algae, macrophytes, epifauna and infauna
Density of macrocarnivorous	Feed on macroinvertebrate and vertebrates (mostly fish)
Density of zooplanktonivores	Feed on planktonic crustacean, hydroids and fish eggs/larvae
Density of herbivorous	Feed predominantly on macroalgae, macrophytes, phytoplankton and microphytobenthos
Mobility	
Density of high mobile individuals	Moved over relatively large distances
Density of individuals with medium mobility	Daily movement patterns on the order of tens of meters
Density of territorial individuals	Limited movement and territorial behaviour
Density of sedentary individuals	Limited moviment and well defined home ranges
Resilience	
Density of individuals with "low" and "very low" resilience	Capacity to recover from changes in the environment; minimum population dubling time: high (up to 1.4 years), medium (1.4 to 4.4 years), low (4.5 to 14 years), very low (more than 14 years) (values available on FishBase)
Density of individuals with "medium" resilience	
Density of individuals with "high" resilience	
Habitat association	
Density of generalist individuals	Generalists species that use all, or most, habitat categories, especially the rocky habitats and less water column and sand
Density of individuals that use water column and cavities	Species using mainly the water column and cavities
Density of individuals using mainly substrate covered by algae and water column	Species using mainly substrates covered by algae and water column (rocky bumps and flats covered by soft algae), these species feed on benthic or encrusting organisms
Density of water column specialists	Water column specialists - feed on planktonic organisms
Density of rock cavity specialists	Rock cavity specialists - mainly used rock cavities, less often rock bumps
Density of rock specialists	Rock specialists - preference for rock bumps and flats, mainly those covered by algae but also bare
Density of sand specialists	Sand specialists - mainly used sand at the bottom of the slopes or on the flats among the rocks
Nursery function	
Density of individuals over maturaty size	Individuals over the maturity sizes (sizes information collected from fishbase)
Density of juveniles	Individuals bellow the maturity size
Biogeographic affinity*	
Density of warm-temperate individuals	Occur from the mediterranean and north-western coasts of Africa to the western entrance of the English Channel
Density of cold-temperate individuals	From north sea to the atlantic coast of Iberian Peninsula (southern limit)
Density of tropical individuals	Range from tropical west Africa to the entrance of mediterranean or the south of Iberian Peninsula

* Only tested for thermal pollution pressure

Different methods can be employed to select the subset of suitable metrics for incorporation in multimetric indices (see Roset et al. 2007 for a review) that should include only those that are: (1) biologically meaningful, (2) able to be reliably and easily quantified using field sampling, (3) sensitive to human disturbance and (4) not redundant with other metrics (Noble et al. 2007; Roset et al. 2007). In order to do so, a Canonical Analysis of Principal Coordinates (CAP, Anderson & Willis 2003) was used to identify the metrics that best discriminate between groups (D vs. C) (Spearman r > |0.5|). The non-parametric Mann-Whitney U test was used to assess whether the metrics of one of the sites (control or disturbed) tend to have larger values than the other (Roset et al. 2007). Finally, Spearman correlations among metrics were used to ascertain the degree to which each pair of metrics was correlated and thus redundant (r > |0.85|). In this way, if a metric was not redundant with others, if it had high correlation with the axes of the discriminant analysis and if their values were consistently higher/lower among replicates of each site, then it was selected.

All the above mentioned analyses, except the Mann-Whitney test and Spearman correlations, were performed per type of pressure in PRIMER 6 with PERMANOVA+ software package. These analyses were based on a Euclidean distance matrix constructed after normalizing each metric by subtracting the mean and dividing by the standard deviation, in order to place all metrics on a comparable measurement scale. P-Values were calculated using 9999 permutations. Mann-Whitney tests and Spearman correlations were carried out using Statistica 10 software. For all analyses the level of statistical significance adopted was 0.05.

In order to improve the interpretation of metric differences, a SIMPER routine was performed to identify the species that contributed most to dissimilarities between each pair of sites (PRIMER 6 software).

Results

Habitat description

The habitats of both sites representing the fishing pressure are characterized by an extensive rocky area (91%) composed by calcareous boulders with different sizes, including some small areas of cobbles (7%) (Mean rugosity ratio: disturbed - 0.2 and control - 0.3). These rocky areas are full of holes, vertical walls and small caves with sponges, polychaetes, hydrozoans, anemones and gastropods recorded in almost all quadrats. The control site was covered by creeping (40%), encrusting algae (15%) and

12% of *Asparagopsis armata* (invasive algae), while the disturbed site was covered predominantly by *A. armata* (66%).

Both habitats associated with sewage pressure are composed by extensive areas of big rocky blocks (88.5%) with vertical walls and some holes surrounded by sand patches (5%) and small areas of cobbles (5%) (Mean rugosity ratio: 0.3). These rocky strata are mainly covered by creeping and encrusting algae (93%). Sponges and polychaetes occur in both sites and gastropods at the control site (recorded in almost all quadrats).

Regarding the pressure of port activities, the habitats consist of natural stone riprap that generates a large number of deep holes (mean rugosity ratio: 0.5). The coverage of these sites was very poor when compared with natural rocky reefs, being characterized by the dominance of creeping and encrusting algae (70%) and a residual presence of invertebrates.

Finally, the site near the thermal effluent comprises extensions of rocky substrate with ridges (83%) and some blocks surrounded by sand (16%) (Mean rugosity ratio: 0.2). The control site had higher algal coverage (92%) characterized by the dominance of creeping and encrusting algae (60%), which contrasted with the 60% of creeping algae in a total of 67% of cover in the disturbed site. Sponges, polychaetes, hydrozoans and gastropods were the dominant invertebrates at both sites.

Fish assemblages

A total of 51 species were identified in a total count of 8983 individuals (Table 2.2). Fish assemblages were characterized by the dominance of species belonging to the families Labridae, Sparidae, Gobiidae and Blenniidae. In general, the species belonging to the Labridae family (except *Coris julis*), and the species *Diplodus sargus*, *D. vulgaris*, *Sarpa salpa*, *Parablennius pilicornis* and *Tripterygion delaisi*, had higher densities at control sites, excluding the family Labridae from the sites inside Sines harbor (Table 2.2). Furthermore, a remarkable difference between fished and protected sites (average dissimilarity: 40.29%) was found for *C. julis*, with 3-fold higher density at the disturbed site and the species *S. salpa* with 2-fold higher density at the control site, each one contributing more than 15% for the dissimilarity (SIMPER results). The remaining species *D. vulgaris, D. sargus*, *T. delaisi and Gobius xanthocephalus* had higher density at the control site (> 5% contribution for sites dissimilarity).

Table 2.2 Mean density (ind. m^{-2}) and standard deviation (in parenthesis) of fish species recorded at control (C) and disturbed (D) sites of the pressures of fishing, port activities, sewage discharges and thermal effluent. * Species with frequency of occurrence lower than 50%.

Species	Fishing		Port activities		Sewage discharges		Thermal pollution	
	C	D	C	D	C	D	C	D
Atherinidae								
Atherina presbyter	*0.05 (0.122)		0.005 (0.005)	*0.002 (0.004)				
Balistidae								
Balistes capriscus			*0.003 (0.005)					
Blenniidae								
Parablennius gattorugine	0.017 (0.015)	*0.01 (0.016)	*0.003 (0.008)		*0.003 (0.008)	*0.007 (0.01)		
Parablennius pilicornis	0.260 (0.076)	0.180 (0.060)	0.723 (0.204)	0.013 (0.016)	0.230 (0.041)	0.077 (0.029)	0.150 (0.078)	0.213 (0.146)
Parablennius ruber		0.013 (0.01)				*0.003 (0.008)		*0.003 (0.008)
Parablennius tentacularis			*0.003 (0.008)					
Callionymidae								
Callionymus lyra			*0.003 (0.008)		*0.003 (0.008)			*0.003 (0.008)
Gadidae								
Pollachius pollachius	*0.002 (0.004)	*0.02 (0.04)						
Gobiesocidae								
Lepadogaster candolii	0.033 (0.045)					*0.007 (0.01)	*0.007 (0.01)	
Lepadogaster lepadogaster	0.040 (0.057)				*0.007 (0.01)	*0.013 (0.024)		
Lepadogaster purpurea	*0.003 (0.008)							
Gobiidae								
Gobius bucchichi					0.010 (0.01)		0.017 (0.015)	0.037 (0.044)
Gobius cobitis	0.020 (0.025)			*0.007 (0.01)				
Gobius cruentatus	0.053 (0.037)		*0.01 (0.024)	0.063 (0.034)				
Gobius niger			*0.01 (0.024)	0.040 (0.052)				
Gobius paganellus	0.020 (0.03)	*0.01 (0.01)	*0.01 (0.024)	*0.01 (0.016)	*0.003 (0.008)	*0.007 (0.01)	0.013 (0.016)	0.027 (0.01)
Gobius xanthocephalus	0.283 (0.210)	*0.01 (0.016)	0.733 (0.228)	0.880 (0.352)	*0.007 (0.016)		0.003 (0.008)	*0.033 (0.037)
Gobiusculus flavescens			*0.003 (0.005)		*0.15 (0.234)		0.123 (0.153)	*0.013 (0.028)
Pomatoschistus marmoratus							0.047 (0.095)	0.090 (0.181)
Pomatoschistus pictus			0.023 (0.029)	0.090 (0.075)				0.070 (0.08)
Labridae								
Centrolabrus exoletus	0.085 (0.071)	0.055 (0.04)		0.015 (0.013)	0.015 (0.019)	0.015 (0.018)		
Coris julis	0.318 (0.178)	0.995 (0.385)			0.317 (0.177)	0.225 (0.098)	0.043 (0.017)	0.040 (0.032)
Ctenolabrus rupestris	0.197 (0.098)	0.160 (0.059)	0.153 (0.112)	0.193 (0.03)	0.163 (0.052)	0.030 (0.027)	0.043 (0.032)	0.137 (0.107)
Labrus bergylta	0.048 (0.017)	0.045 (0.025)		*0.002 (0.004)	0.022 (0.019)	*0.008 (0.013)	0.010 (0.012)	*0.002 (0.004)
Symphodus bailloni	0.015 (0.013)	0.022 (0.021)		*0.003 (0.008)	0.007 (0.008)	*0.003 (0.005)		*0.007 (0.012)
Symphodus cinereus				*0.003 (0.005)				*0.003 (0.005)
Symphodus melops	0.053 (0.045)	0.025 (0.025)	*0.032 (0.023)		0.043 (0.049)	0.018 (0.009)	0.070 (0.04)	0.013 (0.012)
Symphodus roissali	0.053 (0.045)	0.023 (0.017)	0.007 (0.01)		*0.007 (0.01)	*0.005 (0.008)	0.017 (0.019)	0.022 (0.013)
Symphodus rostratus	*0.003 (0.008)							

Table 2.2 (continued)

Species	Fishing		Port activities		Sewage discharges		Thermal pollution	
	C	D	C	D	C	D	C	D
Moronidae								
Dicentrarchus labrax			0.298 (0.134)	0.082 (0.066)		*0.002 (0.004)		
Mullidae								
Chelon labrosus	0.025 (0.027)		0.035 (0.012)	0.010 (0.006)	*0.005 (0.012)			
Liza aurata			0.017 (0.022)	*0.005 (0.012)				
Liza ramada			*0.002 (0.004)	*0.002 (0.004)				
Liza spp.	0.002 (0.004)	*0.002 (0.004)		*0.022 (0.034)	*0.002 (0.004)			
Mugil cephalus	0.013 (0.019)	*0.002 (0.004)						
Mullus barbatus		*0.002 (0.004)						
Mullus surmuletus		*0.002 (0.004)			*0.002 (0.004)			0.012 (0.019)
Scophthalmidae								
Zeugopterus punctatus		*0.003 (0.008)						
Scorpaenidae								
Scorpaena porcus		*0.003 (0.008)	*0.003 (0.008)		*0.01 (0.016)	*0.01 (0.02)		*0.003 (0.008)
Serranidae								
Serranus cabrilla	0.028 (0.027)	0.022 (0.021)			0.005 (0.005)	*0.002 (0.004)		
Sparidae								
Boops boops				0.147 (0.320)	0.092 (0.128)		*0.05 (0.122)	
Diplodus annularis	*0.013 (0.02)							
Diplodus cervinus		0.012 (0.014)	*0.003 (0.004)	*0.003 (0.004)				
Diplodus puntazzo		*0.003 (0.008)	0.005 (0.005)					
Diplodus sargus	0.450 (0.337)	0.355 (0.234)	0.338 (0.183)	0.220 (0.178)	0.042 (0.049)	0.005 (0.005)	*0.005 (0.012)	*0.002 (0.004)
Diplodus vulgaris	0.740 (0.623)	0.702 (0.455)	0.173 (0.144)	0.180 (0.101)	0.183 (0.114)	0.018 (0.018)	0.122 (0.039)	0.052 (0.049)
Diplodus spp.			*0.02 (0.028)					
Oblada melanura	*0.05 (0.108)	*0.005 (0.008)	0.052 (0.059)					
Pagellus acarne		*0.002 (0.004)	*0.005 (0.012)					
Pagellus bogaraveo		*0.003 (0.008)						
Sarpa salpa	1.550 (0.487)	0.772 (0.602)	0.868 (0.722)	0.412 (0.195)			0.010 (0.012)	*0.012 (0.028)
Sparus aurata			0.057 (0.101)					
Spondyliosoma cantharus							*0.007 (0.01)	*0.027 (0.06)
Tripterygiidae								
Tripterygion delaisi	0.437 (0.263)	0.120 (0.043)	0.310 (0.09)	0.283 (0.099)	0.047 (0.041)	0.013 (0.016)	0.080 (0.043)	0.080 (0.035)

When compared with the control site, all species exhibited lower densities at the sewage-disturbed site (Table 2.2), with *D. vulgaris, P. pillicornis, C. julis, Ctenolabrus rupestris, T. delaisi* and *Gobiusculus flavescens* being the highest contributing species for the dissimilarities found (> 5% contribution) (average dissimilarity: 59.48%). Among these species, *C. julis* was the only species that stood out in the sewage-disturbed site, but a great difference in size class distribution was observed: small individuals (< 8 cm) were only recorded at the control site, while adults (> 15 cm) had higher density at the disturbed site.

The main species contributing to the dissimilarities between sites in the case of port activity (average dissimilarity: 43.79%) were *P. pillicornis, S. salpa, Dicentrarchus labrax* and *D. sargus* in the control site and *G. xanthocephalus* in the disturbed site. Finally, for the impact of the thermal effluent (average dissimilarity: 57.04%), only *P. pilicornis* and *C. rupestris* characterized the disturbed site and *G. flavescens*, *Symphodus melops* and *D. vulgaris* the control (> 5% contribution).

Fish-based metrics

PERMANOVA results showed significant differences among the fish metrics for each pair of sites (D vs. C), with the exception of the thermal effluent pressure: fishing (pseudo-F = 3.162, p-value < 0.05), sewage discharges (pseudo-F = 6.594, p-value < 0.05), port activities (pseudo-F = 3.544, p-value < 0.05), thermal effluent (pseudo-F = 1.403, p-value > 0.05). These results were in agreement with the PCO analysis, where the first axis separates disturbed from control sites for all stressors with the exception of the thermal effluent (Figure 2.2). No significant differences in multivariate dispersions were found by the PERMDISP routine within each group (control and disturbed) for all pressures (p-values > 0.05), with the exception of sewage discharges, which differed in their relative dispersion. This is however unlikely to affect the performance of PERMANOVA given the distance between groups (Figure 2.2B).

The results of the discriminant CAP analysis of the fish metrics dataset also showed significant differences between C and D sites (fishing, sewage discharges and port activities with p-values < 0.05). The correlation of individual metrics with the first canonical axis corresponding to pressure effects and the Mann-Whitney test results are shown in Table 2.3 and Table 2.4.

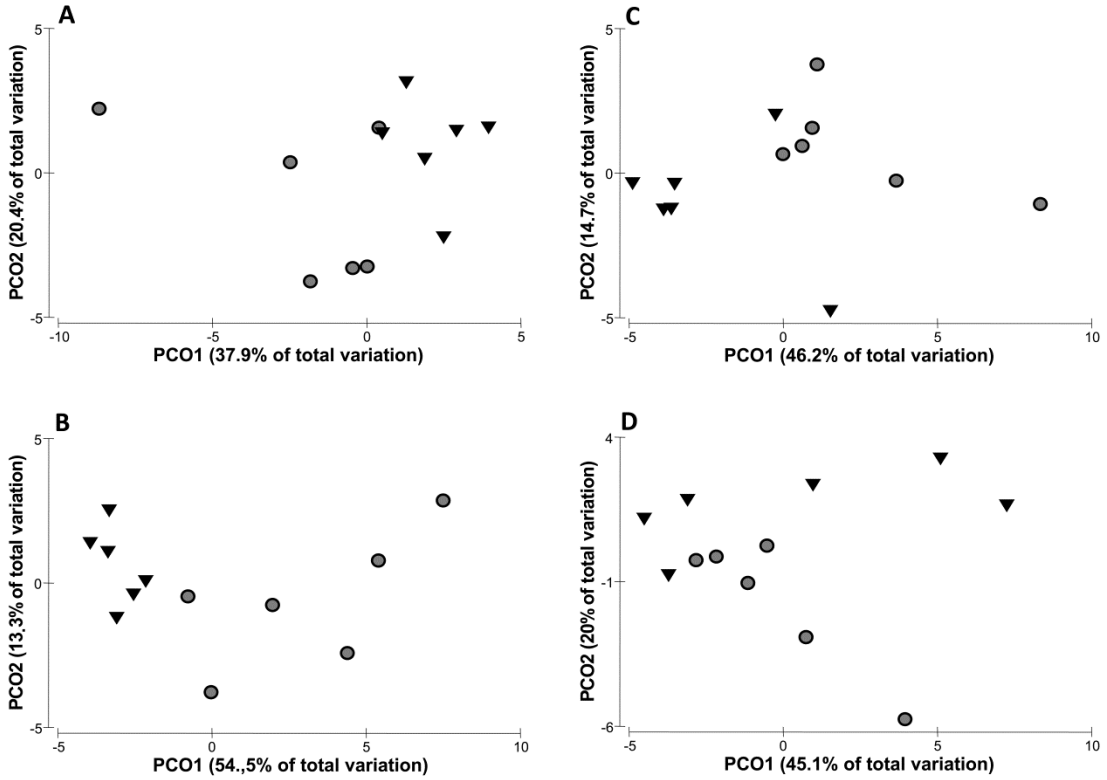

Figure 2.2 Principal Coordinates Analysis (PCO) comparing fish-based metrics at control (gray circles) and disturbed (black triangles) sites for the pressures of fishing (A), sewage discharges (B), port activities (C) and thermal effluent (D).

The highest contributing metrics for the discrimination between the fishing pressure sites were related with the dependence on a specific substrate (density of territorial species, density of rock specialists and density of sand specialists) with r > 0.75 (Table 2.3). These results suggest that habitat features are having a strong influence on species distribution. In fact, if we remove the most habitat-dependent species (Blenniidae, Gobiidae, Gobiesocidae and Tripterygiidae) the effect of fishing pressure is no longer significant (PERMANOVA: Pseudo-F = 1.889 p-value > 0.05). However, the Mann-Whitney test shows that metrics related with medium commercial value and density of herbivores have consistently greater values at the control site, as well as the density of generalist individuals at disturbed site (see Table 2.4). This means that some differences between zones were found but they were not enough to be detected in PERMANOVA. Facing these results and accounting that the metric density of individuals with medium commercial value is highly correlated with several metrics, especially with the density of herbivores (r = 0.90), the metrics density of herbivores and density of generalist individuals were selected.

Table 2.3 Results of the Canonical Analysis of Principal Coordinates (CAP) showing fish-based metrics contributing most to distinguish control from disturbed sites by type of pressure (correlation coefficients r > 0.5). Metrics were calculated using all species. The metrics that showed consistent responses to pressure are indicated (•) (Mann-Whitney test results). Metrics associated with disturbed sites are marked with an asterisk (*).

Fishing		
Density of territorial individuals	0.886	•
Density of rock specialists	0.8194	•
Density of sand specialists	0.7555	•
Total number of species	0.7453	•
Dominance	0.714	•
Density of individuals with medium commercial value	0.6629	•
Density of juveniles	0.6302	
Density of herbivorous	0.6266	•
Density of individuals that use water column and cavities	0.5763	•
Total density	0.5244	
Density of individuals with high resilience	0.5087	
Density of individuals using mainly substrate covered by algae and water column	0.5068	
Density of generalist individuals (*)	-0.7942	•
Sewage discharges		
Density of juveniles	-0.8697	•
Total density	-0.8694	•
Density of individuals that use water column and cavities	-0.8396	•
Density of omnivorous	-0.8367	•
Density of individuals with medium resilience	-0.8344	•
Density of invertebrate feeders	-0.8119	•
Density of rock specialists	-0.789	•
Density of territorial individuals	-0.783	•
Density of individuals with medium mobility	-0.7727	•
Density of high mobile individuals	-0.7726	•
Density of large individuals with medium to high commercial value (> 20 cm)	-0.7513	•
Density of individuals with high resilience	-0.738	•
Density of individuals with high commercial value	-0.6999	•
Density of individuals using mainly substrate covered by algae and water column	-0.6915	•
Commercial/non-commecial ratio (in density)	-0.6752	•
Density of individuals with medium commercial value	-0.5729	
Density of sand specialists	-0.5637	
Port activities		
Density of individuals over maturaty size	-0.9353	•
Density of macrocarnivorous	-0.9301	•
Density of territorial individuals	-0.9164	•
Density of generalist individuals	-0.8798	•
Density of large individuals with medium to high commercial value (> 20 cm)	-0.8655	•
Density of individuals with high resilience	-0.8565	•
Density of rock specialists	-0.8465	•
Total density	-0.7693	
Density of individuals with high commercial value	-0.7499	•
Density of herbivorous	-0.6451	
Density of individuals with medium mobility	-0.6445	
Density of omnivorous	-0.5835	
Density of individuals with medium resilience	-0.555	
Density of juveniles	-0.5075	
Density of individuals with medium commercial value	-0.5046	
Density of invertebrate feeders (*)	0.5467	
Density of sedentary individuals(*)	0.5906	

Table 2.4 Results of the Canonical Analysis of Principal Coordinates (CAP) showing fish-based metrics contributing most to distinguish fished from control sites, estimated without cryptobenthic species (correlation coefficients |r| > 0.5). Metrics that showed consistent responses to pressure are marked (•) (Mann-Whitney test results). Metrics associated with disturbed sites are marked with an asterist (*).

Fishing	
Density of individuals with medium commercial value	0.9374 •
Density of herbivorous	0.9242 •
Density of juveniles	0.8437
Density of individuals with medium mobility	0.7491
Commercial/non-commecial ratio (in density)	0.6579 •
Density of individuals with medium resilience	0.6505
Density of individuals using mainly substrate covered by algae and water column	0.6285
Density of large individuals with medium to high commercial value (> 20 cm)	0.5136
Density of generalist individuals (*)	-0.5677 •
Density of sand specialists (*)	-0.5753

On the other hand, despite not being consistently higher at the control site, the metric density of large individuals with medium to high commercial value (> 20 cm) was selected, since it had lower correlation with other metrics and has a greater potential as indicator of fishing pressure.

For the remaining pressures, the PERMANOVA results without cryptobenthic species were consistent with the ones previously obtained, maintaining the differences between sites (C vs. D) for the sewage and port activities and showing non-significant results for the thermal effluent. There was a greater number of metrics responding to sewage discharges and port activities and the majority of them tend to have higher values at the control site. The density of juveniles and total density were the highest contributing metrics for the discrimination between the sewage and the control with r > 0.85. While the density of individuals over maturity size, density of macrocarnivores, density of territorial individuals and density of generalist individuals were associated with the control site of port activities (see Table 2.3 for details of remaining metrics). Through the analysis of the Spearman rank correlations, the above mentioned metrics related with trophic guilds and the metrics density of juveniles, density of territorial individuals and density of generalist individuals were selected due to their lower redundancy and higher discriminating response between control and disturbed sites. In this context, two major patterns of response were identified: (1) selective pressure, which affects differentially the fish assemblages, with specific metrics responding to pressure (fishing); and (2) broad-range pressure, which affects the entire fish assemblage with fish metrics representing several attributes (trophic, structure, resilience, habitat, nursery function) responding to its presence, independently of the assemblage type (sewage discharges, port activities).

Summarizing, in a multi-stressor approach perspective and accounting for sensitivity, biological meaning and redundancy results, the following metrics were selected as the most suitable to predict and understand fish assemblage changes: density of invertebrate feeders, density of omnivores, density of macrocarnivores, density of herbivores, density of generalists, density of territorial individuals, density of large individuals with medium to high commercial value (> 20 cm) and density of juveniles.

Discussion

Since ecological guilds group species with some degree of functional overlap, the metric approach provides an operational unit linking individual species characteristics with community level responses (Noble et al. 2007) and, as showed in this study, is extremely useful to understand changes due to human-induced pressure.

Effects of fishing

Although the choice of a control site to represent the fishing pressure attempted to maximize de structural similarity among sites, the diversity of boulder sizes was greater at the control site than at the fished site (Gonçalves et al. 2002). Random-sized boulders constitute a complex habitat (Gonçalves et al. 2002) and consequently tend to support higher density and diversity of cryptobenthic species (Macpherson 1994; Fasola et al. 1997; La Mesa et al. 2006). Additionally, canopy formed by *A. armata* (dominant at fished site) creates refuge that may reduce predation by fishes on small epifauna (Sala 1997). Although these algae present clear seasonal variability (Sala 1997), the observed fish assemblage does not change with the reduction of its cover (Henriques et al. unpublished data). Therefore, considering the low mobility of territorial cryptobenthic species, their diet of small invertebrates and habitat complexity could explain their preference for the control site. These facts clarify the results of discriminant analysis, where the metrics that best explained the differences between sites were associated to cryptobenthic species.

Increases in total abundance, biomass and size of fish within the boundaries of MPA of Arrábida, particularly for target species, are some of the reported differences as a result of protection (see García-Charton et al. 2008 for a review). Besides, changes in abundance of predatory fish can cause ecosystem wide effects such as trophic cascades (Pennigar et al. 2000; Guidetti & Sala 2007) and decreases in small cryptic fishes (Willis & Anderson 2003), which make the effects stemming from fishing very complex. Furthermore, these differences increase with time of protection and are dependent of other factors such as

recruitment patterns, exportation of biomass, connectivity, fishing effort outside the reserve, law enforcement and reserve size (Claudet et al. 2006; García-Charton et al. 2008; Guidetti et al. 2008). This MPA was established in 2005 but a phased process to implement the regulatory measures of the no-take area was adopted. Although the control site was located in an area that is fully protected since 2008, the 2 years of protection were not enough to detect strong differences between sites (C vs. D) regarding the fish-based metrics estimated with the non-cryptobenthic species, suggesting that this reserve is still on a trajectory of recovery.

In general, Sparidae, Serranidae and large Labridae individuals appear to benefit from protection, however, the response patterns of fishes remains heterogeneous (García-Charton et al. 2008). The results of some meta-analyses are in agreement with the observed pattern, suggesting that the species *S. salpa* responds positively to medium to high levels of protection (Guidetti et al. 2008), while *C. julis* has a negative response (Ojeda-Martinez et al. 2007). Such results were associated to different factors like density of predators, local fishing traditions and inter-specific relationships (Ojeda-Martinez et al. 2007; Guidetti et al. 2008). *C. julis* is a generalist species (wide home ranges and flexibility of diets) without commercial value in this zone, so the high density observed at the fished site is probably a consequence of competition with other species that take advantage of protection. On the other hand, the highest densities of the herbivore *S. salpa* are probably directly related with fishing impacts, considering that it is frequently caught by fishing gears used in this area and that the same density pattern was observed in other seasons when *A. armata*, that is not in the diet of this species, was much less abundant at the fished zone (Henriques et al. unpublished data). These facts explain the CAP analysis and Mann-Whitney results, where the density of herbivores, density of individuals with medium commercial value and density of juveniles characterized the control site, while, the density of generalist individuals was consistently associated to fished sites.

Slightly higher densities of the target species *Diplodus* spp. were found at control site but their late maturity, slow growth rate and low rates of recruitment makes their recovery longer (Ojeda-Martinez et al. 2007). Although not always consistently higher at the control site, the metric density of large individuals with medium to high commercial value (> 20 cm), composed mainly by Sparidae and some large Labridae individuals (*Labrus* spp.), helps in the discrimination between sites (C vs. D). These results are in accordance with previous work by Claudet et al. (2006), that only found significant differences in medium-sized species (20-30 cm) and in medium to high-value commercial species after 3 years of protection, as well as for the large species (> 30 cm) after 6 years. Regarding the

remaining metrics, it was expected that the number of species, the total density and the density of macrocarnivores would have higher values at the control site (Claudet et al. 2006; Guidetti & Sala 2007; Guidetti et al. 2008). However, the number of species metric loss significance when the most habitat-dependent species were removed from the analyses and the total density metric was not significant probably due to the replacement of species (e.g. *C. julis*). Finally, as the density of macrocarnivores is composed by relatively uncommon and usually solitary species with slow growth rates (e.g. *Conger conger*, *Muraena helena*, *Serranus cabrilla*) their response to fishing protection is delayed. This means that with larger protection periods these metrics could become good indicators, nonetheless, the metric density of large individuals with medium to high commercial value (> 20 cm) seems more sensitive, since it takes into account both density and size of species directly impacted by fishing.

Effects of sewage discharges

Urban and industrial untreated discharges led to broad range changes on fish assemblages, with significant differences observed between control and disturbed sites using fish-based metrics with and without cryptobenthic species. These results suggest that the whole fish assemblage was affected by the sewage discharges pressure. Changes on diversity, abundance and trophic structure of fish assemblages are some of the main responses reported due to sewage impacts (McKinley & Johnston 2010 and references therein). However, the direction of changes is often unclear. For instance, the effect on species richness is controversial, with reports of increase, decrease or even no differences found between control and disturbed sites (e.g. Guidetti et al. 2002; Khalaf & Kochzius 2002; Guidetti et al. 2003; Johnston & Roberts 2009; Azzurro et al. 2010; McKinley & Johnston 2010). In fact, the metric total number of species had slightly higher values at the control site and low correlation with canonical axes ($r = 0.44$). Consequently, it was considered a weak indicator of sewage effects.

The main differences between C and D sites were in the density of juveniles, total density, density of individuals that use water column and cavities, density of omnivorous, density of species with medium resilience and density of invertebrate feeders. These results are explained by strong differences in density of Sparidae (omnivorous), Labridae (invertebrate feeders, medium resilience) and some cryptobenthic species (omnivorous and invertebrate feeders) which were higher at the control site. A decrease in abundance of Sparidae and Labridae due to sewage was previously demonstrated (Guidetti et al. 2002; Guidetti et al. 2003; Azzurro et al. 2010). In the present study, 3-fold higher density was observed for the

cryptobenthic species *P. pilicornis* and *T. delaisi* at the control site, which contrasts with results from Azzurro et al. (2010), who found higher abundances of opportunistic-tolerant cryptobenthic species (*Gobius buchicchi* and *Parablennius rouxi*) at sewage impacted locations. Due to their lower mobility and high dependence on the substratum, benthic fishes are probably more affected by contaminants, unless they profit from opportunistic life-history strategies or high tolerance to stressful conditions (Azzurro et al. 2010). Despite the organic enrichment expected from the sewage discharge, the disturbed site is also possibly influenced by industrial wastewaters coming from a nearby stream (personal observations), which could explain not only the low density of the above-mentioned species and juveniles but also the low total density at the disturbed site, except for adults of *C. julis*. In fact, a general decrease in fish abundance (~50%), invertebrate and fish feeders and young life stages (larvae, settlers and juveniles) were observed as a response to industrial disturbance (Khalaf & Kochzius 2002; McKinley & Johnston 2010). Moreover, generalist species like *C. julis* that have physiological mechanisms and develop cytoprotective proteins that increase their tolerance to pollutants, could profit from these impacted areas (Fasulo et al. 2010), explaining the fact that it was the only species abundant at the disturbed site.

While it has been demonstrated that some trophic guilds benefit from sewage plumes (detritivores and planktivores) (Guidetti et al. 2002; Guidetti et al. 2003; Azzurro et al. 2010), even in association with industrial pollution (Khalaf & Kochzius 2002), none of them stood out at the disturbed site. This contradictory result is probably due to weak detection of pelagic species (e.g. Mugilidae) with the sampling method used that is necessarily directed at demersal fish assemblages at depths close to 10m (deep stratum). Unfortunately, the poor visibility of mainland Portuguese waters reduces the effectiveness of sampling methods for pelagic species (such as stationary points). These facts also explain the differences obtained for the total density metric in relation to other studies, since the higher total abundance observed at sewage-impacted sites in those studies are related to high abundances of detritivores and planktivores (Guidetti et al. 2002; Guidetti et al. 2003; Azzurro et al. 2010).

Effects of port activities

Little is known about the impacts of port activities on fish assemblages because research carried out has focused on the effects of artificial substratum (e.g. Clynick, 2006, 2008; Pizzolon et al., 2008; Wen et al., 2010). Furthermore, two studies performed in zones influenced by several anthropogenic pressures, including port activities, reported a general

decrease in fish abundance despite not finding significant differences in the number of species (Khalaf & Kochzius 2002; Järvik et al. 2005).

Like with sewage discharges, the whole fish assemblage was affected by this pressure, which led to broad range changes on several metrics. In fact, the metric differences found between these pressures are only related to the assemblage type that is affected. In general, fish assemblages associated with artificial substrates are characterized by species with large mobility and few sedentary/territorial species as well as low abundance or absence of common reef-associated species (e.g. Clynick 2006, 2008; Pizzolon et al. 2008; Wen et al. 2010). This explains why metrics related to size, generalist species, top predators (macrocarnivores) and some territorial/sedentary low mobile species were the most sensitive (see table 2.3 for details). Moreover, similarly to what happened with sewage effluent, total density and density of juveniles were higher at the control site, probably due to higher levels of pollution at the disturbed site. Indeed, activities associated with marinas, including boat cleaning, leakage of fuel and organic waste disposal cause pollution (Clynick 2006 and references therein), which in this case is intensified by the presence of the neighbouring fishing port highly contaminated by chemical, microbiological and organic compounds. Furthermore, it has been reported that marinas have an important nursery function for many commercial species (e.g. Sparidae) (Clynick 2006), so in this case the sensitivity of juveniles to pollution makes the metric density of juveniles extremely important to assess this type of pressure.

Effects of thermal effluent

Since the sampled sites of the thermal effluent pressure are located in temperate waters, it is expected that the majority of species can tolerate a broad range of temperatures, with few species living near their tolerance limits. With exception of the metric density of cold-temperate individuals (due to the gregarious species *G. flavescens*), no other fish-based metrics were specifically linked with the thermal effluent. Thus, the increase of 1ºC (observed difference between C and D sites at the bottom) was not enough to produce significant changes on fish assemblages, showing that the functional approach is strategic to detect assemblage changes at both structural and functional levels, despite the small differences found for a few species. This result is in accordance with observations by Teixeira et al. (2012) for fish and other biological groups, where no differences were found with an increase of 2ºC in tropical waters (supposedly more sensitive to thermal stress).

Metrics selection

Fish-based metrics were selected according to their sensitivity and biological meaning, as well as their redundancy with other metrics. This way, all the metrics of trophic structure (except for zooplanktivores) and the metrics density of territorial individuals, density of generalist individuals, density of large individuals with medium to high commercial value (> 20 cm) and density of juveniles, were chosen as the most suitable to assess changes on temperate reef fish assemblages. Conversely, although broadly used in estuarine and freshwater fish-based indices, the metrics number of species and dominance were not selected as they showed weak responses to the studied pressure sources. In general, attributes with few functional guilds (composed by 2-3 metrics) have higher redundancy than other metrics (e.g. attribute of resilience). Moreover, the response patterns of metrics measured in ratio are difficult to predict since they are dependent of the observed total density (e.g. commercial/non commercial ratio).

There is some disagreement about the extent to which redundancy among metrics is problematic for developing multimetric indices. Despite not having been included in the final selection due to some degree of redundancy with other metrics, the total density metric provides sensitive information to detect pollution problems. Thus, future research regarding the implications of selecting redundant metrics that are highly sensitive to pressures and the test of methods to prevent metric overfitting (e.g. down weighting redundant metrics so that they count as a single metric in the final index value) are required in order to conclude if the use of these metrics is suitable.

Some of the existing multimetric indices employ metrics related with opportunistic species (Noble et al. 2007). From our results, the possible use of adults of *C. julis* as indicator species for degraded sites (tolerant to pollution and generalist) seems promising but care is needed since this species prefers deeper habitats (García-Charton & Pérez-Ruzafa 2001). Furthermore, no assessment of quality status of fish communities should be made without looking for species information to check if some tolerant-opportunistic species are affecting the results and consequently leading the metric values in unexpected directions (e.g. increase of opportunistic-tolerant cryptobenthic individuals at sewage impacted locations).

The results obtained in this study highlight the importance of having species-habitat relationships into consideration when interpreting metric values, in order to ensure that differences found are due to the presence of a human-induced pressure. This is especially important in control-impact sampling designs which are the most commonly used, as many

times before-impact data is not accessible or the sampling methods are not comparable (Osenberg et al. 2006). Finally, the use of replicate variability to test metrics strengthened the sensitivity and consistency of the selection, considering that the application of multimetric indices often uses the sum of replicates to calculate final metric values.

Although it was possible to select a group of sensitive metrics, further research is needed to address spatial (including across biogeographic regions) and temporal (seasonal and inter-annual) variability in the response of those metrics. Moreover, this research should include not only the assessed pressures but also other drivers of pressure (e.g. dredging activities, aquaculture), in order to test the applicability of the selected metrics and strengthen their sensitivity. Overall, the results obtained were supported by other studies that analyzed the effects of similar pressures at the species level, thus the use of the selected metrics seems promising. However, it would be premature to reach a final conclusion regarding their use in multimetric indices without further testing, and this study is but a starting point for the successful use of reef fish assemblages as indicators.

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Supplementary data I. Database used to calculate the several fish-based metrics. The exhibit list present the ecological parameters characterized for each species: trophic level, maturity length, mobility (hm - high, mm - medium, te - territorial, se - sedentary), feeding guilds (inv - invertebrate feeders, ma - macrocarnivores, pi - piscivores, om - omnivores, zoo-zooplanktonivores, he-herbivores), qualitative abundance (VC - very common, C - common, LC - less common, R - rare), commercial value (€ - nor or low, €€ - medium, €€€ - high), resilience (VL - very low, L - low, M - medium, H - high), habitat association (rockcave - use mainly substrate covered by algae and water column, watcave - use water column and cavities, gen - generalists individuals, rockspe and sandspe - rock or sand specialists, respectively, watalgae - use mainly substrate covered by algae and water column, wat - water column), biogeographic group (Temp- temperate, Eury- eurythermic, Warm - warm-temperate, Cold- cold-temperate, Trop- tropical).

Species	Trophic level	L maturity (cm)	Mobility	Feeding guilds	Qualitative abundance	Commercial value	Resilience	Habitat association	Biogeographic group
Atherina presbyter	3.7	9.7	hm	ma	VC	€	H	wat	Temp
Balistes capriscus	3.5	29.5	hm	inv	C	€€	H	watalgae	Eury
Parablennius gattorugine	2.9	18.5	te	om	VC	€	H	rockspe	Warm
Parablennius pilicornis	3.2	8.6	te	om	VC	€	H	rockspe	Trop
Parablennius ruber	2.9	8.5	te	om	C	€	H	rockspe	Temp
Parablennius tentacularis	3.1	10	te	om	UC	€	H	rockspe	Warm
Callionymus lyra	3.3	15	mm	inv	C	€	M	sandspe	Temp
Pollachius pollachius	4.2	67.5	hm	inv	C	€€€	M	gen	Cold
Lepadogaster candolii	2.8	5.4	te	inv	C	€	M	rockcave	Warm
Lepadogaster lepadogaster	3.3	4.8	te	inv	C	€	M	rockcave	Warm
Lepadogaster purpurea	3.3	5.4	te	inv	UC	€	M	rockcave	Temp
Gobius bucchichi	3.1	7	te	om	C	€	M	sandspe	Trop
Gobius cobitis	3	15.2	te	om	VC	€	M	rockspe	Warm
Gobius cruentatus	3.1	11.8	te	om	VC	€	M	sandspe	Warm
Gobius niger	3.2	11.5	te	om	VC	€	M	rockspe	Temp
Gobius paganellus	3.3	8.8	te	om	VC	€	M	rockspe	Warm
Gobius xanthocephalus	3.1	7	te	om	VC	€	H	sandspe	Warm
Gobiusculus flavescens	3.2	4.5	mm	zoo	VC	€	H	watalgae	Cold
Pomatoschistus marmoratus	3.3	5.8	se	inv	VC	€	H	sandspe	Warm
Pomatoschistus pictus	3.1	4.5	se	inv	VC	€	H	sandspe	Temp

Species	Trophic level	L maturity (cm)	Mobility	Feeding guilds	Qualitative abundance	Commercial value	Resilience	Habitat association	Biogeographic group
Centrolabrus exoletus	3.5	8.5	mm	inv	VC	€	H	watcave	Cold
Coris julis	3.2	16.2	mm	inv	VC	€	M	gen	Temp
Ctenolabrus rupestris	3.3	10.1	mm	inv	VC	€	M	watcave	Temp
Labrus bergylta	3.1	31.9	mm	inv	VC	€€	L	watalgae	Cold
Symphodus bailloni	3.3	12.9	mm	inv	VC	€	M	rockspe	Temp
Symphodus cinereus	3.3	9.8	mm	inv	VC	€	M	rockspe	Warm
Symphodus melops	3.3	13.1	mm	inv	VC	€	M	rockspe	Cold
Symphodus roissali	3.5	10.4	mm	inv	VC	€	M	rockspe	Warm
Symphodus rostratus	3.4	7	mm	inv	C	€	H	rockspe	Warm
Dicentrarchus labrax	3.8	44.4	hm	ma	VC	€€€	M	gen	Temp
Chelon labrosus	2.4	38.1	mm	om	VC	€€	M	wat	Temp
Liza aurata	3.1	37.3	hm	om	VC	€€	M	wat	Temp
Liza ramada	2.3	33	hm	om	VC	€€	M	wat	Temp
Mugil cephalus	2.1	54.6	hm	ma	VC	€€	M	wat	Trop
Mullus barbatus	3.2	15.5	mm	inv	VC	€€€	M	sandspe	Temp
Mullus surmuletus	3.4	26.2	hm	inv	VC	€€€	M	sandspe	Temp
Scorpaena porcus	3.9	16.4	se	ma	VC	€€	M	rockcave	Warm
Serranus cabrilla	3.3	13.6	mm	ma	VC	€	M	watcave	Trop
Boops boops	3	19.8	hm	om	C	€€	M	watalgae	Eury
Diplodus annularis	3.4	16	mm	om	C	€€€	M	watalgae	Warm
Diplodus cervinus	3	31.6	hm	om	C	€€€	L	watalgae	Warm
Diplodus puntazzo	2.9	34.1	hm	om	C	€€€	M	watalgae	Trop
Diplodus sargus	3	21	hm	om	VC	€€€	M	watalgae	Trop
Diplodus vulgaris	3.2	16.5	hm	om	VC	€€€	H	watalgae	Warm
Oblada melanura	3	20.6	hm	om	C	€€	M	watalgae	Trop
Pagellus acarne	3.5	17.7	hm	ma	VC	€€€	M	gen	Temp
Pagellus bogaraveo	3.5	33.6	mm	ma	C	€€€	L	gen	Temp
Sarpa salpa	2	28	mm	he	VC	€€	M	watalgae	Trop
Sparus aurata	3.4	31.9	mm	om	VC	€€€	M	gen	Warm
Spondyliosoma cantharus	3.3	31.1	hm	om	VC	€€	M	watalgae	Temp
Tripterygion delaisi	3.4	6.3	te	inv	VC	€	H	rockspe	Warm

CHAPTER 3

Henriques S., Pais M.P., Costa M.J. & Cabral H.N. (2013). Seasonal variability of rocky reef fish assemblages: detecting functional and structural changes due to fishing effects. Journal of Sea Research 79, 50-59.

Seasonal variability of rocky reef fish assemblages: Detecting functional and structural changes due to fishing effects

Abstract: The present study analysed the effects of seasonal variation on the stability of fish-based metrics and their capability to detect changes in fish assemblages, which is yet poorly understood despite the general idea that guilds are more resilient to natural variability than species abundances. Three zones subject to different levels of fishing pressure inside the Arrábida Marine Protected Area (MPA) were sampled seasonally. The results showed differences between warm (summer and autumn) and cold (winter and spring) seasons, with the autumn clearly standing out. In general, the values of the metrics density of juveniles, density of invertebrate feeders and density of omnivores increased in warm seasons, which can be attributed to differences in recruitment patterns, spawning migrations and feeding activity among seasons. The density of generalist/opportunistic individuals was sensitive to the effect of fishing, with higher values at zones with the lowest level of protection, while the density of individuals with high commercial value only responded to fishing in the autumn, due to a cumulative result of both juveniles and adults abundances during this season. Overall, this study showed that seasonal variability affects structural and functional features of the fish assemblage and that might influence the detection of changes as a result of anthropogenic pressures. The choice of a specific season, during warm sea conditions after the spawning period (July-October), seems to be more adequate to assess changes on rocky-reef fish assemblages.

Keywords: Seasonal variability, fish-based metrics, fishing pressure, temperate rocky reefs, Portugal.

Introduction

Temperate rocky reefs are characterized by a large biological diversity which depends on the interaction of physical and biological factors that can cause strong fluctuations in the distribution and abundance patterns of marine assemblages (Holbrook et al. 1994; Rubal et al. 2011). Habitat complexity and exposure (e.g. Friedlander & Parrish 1998; Lara & Gonzalez 1998; García-Charton & Pérez-Ruzafa 2001; Friedlander et al. 2003; La Mesa et al. 2011a), seasonal variability (e.g. Holbrook et al. 1994; Friedlander & Parrish 1998; Magill & Sayer 2002; Beldade et al. 2006) and inter-annual climatic shifts (e.g. Holbrook et al. 1994; Henriques et al. 2007), are some of the main factors affecting the persistence of species in reefs depending on their ecological requirements, interspecific relationships, life-cycle and mobility patterns. Consequently, they can buffer the effects of anthropogenic pressures or lead to misinterpretation of changes in marine communities (Holbrook et al. 1994).

In recent years, short-term indicators of anthropogenic effects on marine assemblages have become an important issue in applied ecology and implementation of international policies, such as the Marine Strategy Framework Directive (Directive 2008/56/CE). The

usefulness of any state indicator will depend on how well it is able to distinguish anthropogenic from natural variability (Niemi & McDonald 2004). Thus, the analysis of any indicator should take into consideration the above-mentioned factors in order to not only understand stress-response relationships, but also to select the most suitable indicators.

Marine Protected Areas (MPA) are broadly used in marine conservation, aiming to restore and protect the structure and function of marine ecosystems (Micheli et al. 2004). By limiting or forbidding fishing activities in some areas (e.g. no-take zones), MPAs are the best case studies to analyse the effects of fishing on multispecies assemblages as well as their recovery trajectories (Micheli et al. 2004). Although the differences between protected and fished areas depend upon the age of reserves, several other factors could contribute to reserve effectiveness, namely law enforcement, species home-range, fishing effort outside the reserve, reserve size, species recruitment patterns and connectivity between habitats (Claudet et al. 2006; García-Charton et al. 2008; Guidetti et al. 2008). Moreover, these factors could lead to differences between geographical areas in what regards the effects of fishing, making them less predictable.

In general, MPAs are expected to increase the density and biomass of fish assemblages, especially of target and large-bodied species, nevertheless, several studies report complex top-down and bottom-up changes due to habitat quality improvement, competition and predator-prey interactions, which can lead to changes on non-target species depending upon their role in the ecosystem (e.g. Pennigar et al. 2000; Ruitton et al. 2000; Willis & Anderson 2003; Micheli et al. 2004; Guidetti & Sala 2007). In Europe, MPA effects are well known for some geographical areas like the Mediterranean Sea (see García-Charton et al. 2008 for a brief review; Fenberg et al. 2012), but are poorly studied in the north-eastern Atlantic. Although several fish-based metrics related with the abundance/biomass of trophic groups (e.g. piscivores and macrocarnivores), fish size, high commercial value and indicator species have been successfully used as indicators of fishing pressure within MPAs (e.g. Claudet et al. 2006; Guidetti & Sala 2007), there is a considerable lack of knowledge about the consequences of seasonal variability on those metrics, since in this case the studies have been focused on seasonal fluctuations of species (e.g. Holbrook et al. 1994; Friedlander & Parrish 1998; Magill & Sayer 2002; Beldade et al. 2006).

It is recognized that the use of functional guilds to assess anthropogenic impacts has several advantages as they tend to be more resilient to natural variation and respond more predictably to stress (Elliott et al. 2007; Henriques et al. 2008; Pais et al. 2012). Furthermore, since guilds group species with some degree of functional overlap in the

ecosystem they could be easily applicable to other regions (Elliott et al. 2007; Noble et al. 2007). In this context, based on several fish-based metrics (guild approach), the present study aimed to assess structural and functional changes in fish assemblages due to fishing pressure and to understand the influence of seasonal variability on those metrics as well as their ability to detect changes.

Material and Methods

Study area

The Arrábida MPA (mainland Portugal; NE Atlantic) was formally established in 2005 but the regulatory measures were gradually implemented over the following four years (see Sousa 2011 for details of implementation process). The before MPA covers about 53 km^2 which includes a total protection zone (TPZ) of 4 km^2, four partial protection zones (PPZ) and three complementary protection zones (CPZ) covering 21 km^2 and 28 km^2, respectively (Figure 3.1). Regarding the commercial fishing inside this MPA, only licensed vessels under 7 m length are allowed to fish, but with several restrictions: in the CPZ, fishing activities with traps, jigging, longline and handline are allowed in all areas, whereas nets are permitted only farther than 1/4 nm from shore line; in the PPZ, only traps, jigging and handline are allowed farther than 200 m from shore line; the TPZ is a "no-take-no-entry" area; in the whole MPA, trawling, dredges and handcathing commercial fishing are forbidden. Finally, recreational angling is only permitted in the CPZ while spearfishing is forbidden in all zones.

At the time of this study, about 73 vessels operated inside the MPA, but the number of angling fishermen is unknown since anyone with a recreational fishing license can fish in the CPZ. Fishing effort is high in all fished zones, being concentrated in the CPZ near the Sesimbra village and in the PPZ surrounding the TPZ (Cabral et al. 2008). According to Cabral et al. (2008), traps are one of the most commonly used gears within the MPA, while gill and trammel nets concentrated in the CPZ near the exit of the Sesimbra port (> 1/4 nm) as well as vessels fishing with jigs, longlines and handlines that normally operate less than 200 m from shoreline (see Figure 3.1 for fishing pressure details). It is important to note that the values of fishing pressure in figure 3.1 correspond to a normal fishing day and were estimated between September 2007 and February 2008, just before the end of fishing allowance in the TPZ. This data represents the best available information to date about the fishing effort within the MPA.

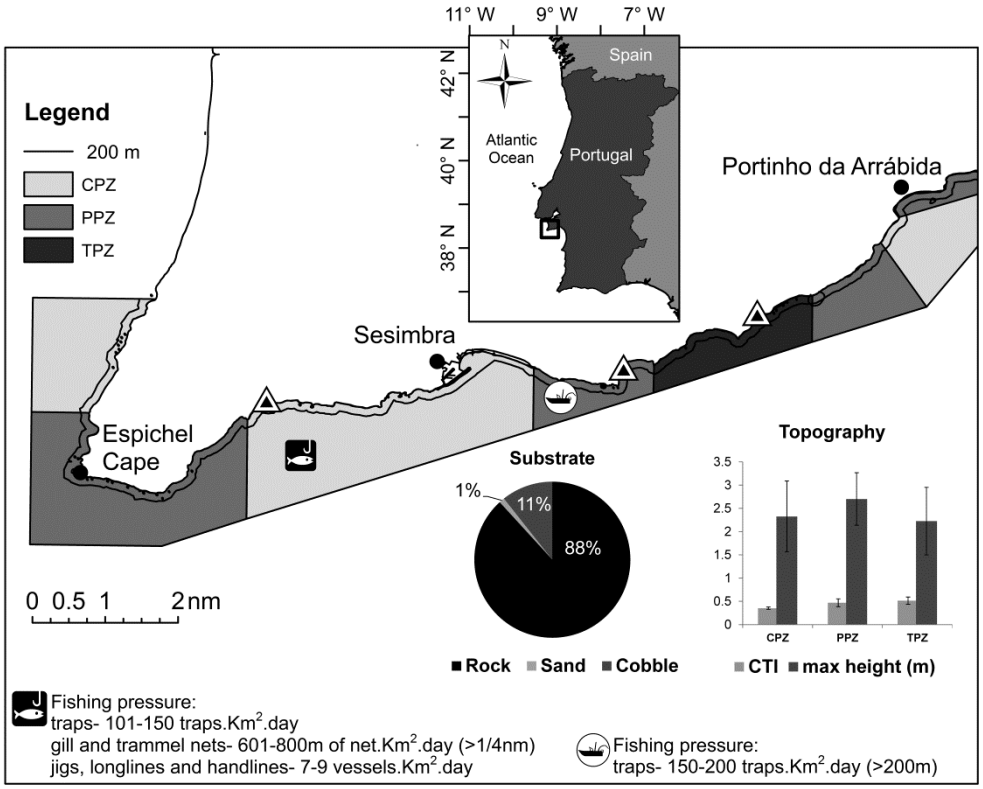

Figure 3.1 Location of the Arrábida MPA showing the different levels of protection: CPZ - complementary protection zones, PPZ - partial protection zones, TPZ - total protection zone. Triangles indicate sampled sites. Information about habitat complexity of the sampled sites is shown in the graphs. Fishing pressure values were based on the previous study of Cabral et al. (2008).

This MPA faces south and is therefore protected from the prevailing north and northwest winds and waves (Gonçalves et al. 2002; Henriques et al. 2007). Subtidal rocky habitats are highly heterogeneous, resulting from the disintegration of calcareous cliffs that border the coastline and extend to tens of meters (Gonçalves et al. 2002; Henriques et al. 2007). These rocky areas are composed of mixed patches of sand, gravel, cobble, random-sized blocks and bedrock. Due to their heterogeneity and topographic complexity, they support a large number of fish species (Gonçalves et al. 2002; Beldade et al. 2006).

To analyse the effects of fishing on rocky reef fish assemblages, three zones were selected inside the MPA, one at each protection level (TPZ, PPZ, CPZ) (Figure 3.1). These zones were selected based on their habitat characteristics which are of similar complexity. The Combined Topography Index (CTI), the percent cover of rock, cobble and sand, the cover of algae by structural groups (i.e. creeping, encrusting, tufts, sheet and filamentous) and the presence/absence of invertebrate groups (i.e. sponges, anemones, hydrozoans, gorgonians, polychaetes, gastropods, crustaceans, sea urchins, star-fish, sea cucumbers

and ascidians) were used to characterize habitat complexity. Habitat sampling was performed by depth strata, by deploying 12 quadrats (50x50 cm) to estimate algal cover and the presence of invertebrates, and 8 replicates of the chain-and-tape method for the remaining measures, by using a 25 m leaded rope as a chain and a 25 m measuring tape to calculate the linear distance travelled by contouring the chain over the substrate and to estimate the percent cover of different substrates (see Pais et al. 2013 for details). Since topography remains similar year round, only quadrat sampling was repeated seasonally. CTI was estimated for each chain-and-tape replicate through the formula CTI = (1-SR) + NC/25 + MVR/25, where SR is the substrate rugosity index, NC the number of corrugations and MVR the maximum vertical relief in meters (see Pais et al. 2013). The average value of the CTI among replicates of both depth strata was used to characterize each sampled zone.

Fish assemblages

Fishes were seasonally sampled at each site from May 2010 to February 2011 using underwater visual census methods. Based on a pilot study (see Henriques et al. 2013), 50 m long strip-transects were randomly placed parallel to the coastline at two depth strata (0−5 m and 5−10 m). Each transect was inspected twice, first pass for demersal species (50x2 m) and the second for cryptobenthic species (50x1 m). On the cryptobenthic pass only the families Blenniidae, Bothidae, Batrachoididae, Callionymidae, Congridae, Gadidae (subfamilies Phycinae and Lotinae), Gobiesocidae, Gobiidae, Muraenidae, Scorpaenidae, Scophthalmidae, Soleidae, Syngnathidae, Tripterygiidae and the species *Ctenolabrus rupestris* and *Labrus mixtus,* as well as *Symphodus* spp. with less than 5 cm total length, were counted (Henriques et al. 2013). A total of 144 transects were performed, corresponding to six replicates per zone and per season. Each replicate included observations for both depth strata pooled together, i.e. one transect at a 0-5 m depth range and another at a 5-10m range, performed in the same dive (~80 min). A total of two replicates were done per day. Transects were performed with a minimum visibility of 5 m. In all transects, the abundance and total length of fish were recorded by the same divers (S. Henriques or M.P. Pais) in order to minimize observer effects.

All fish species were allocated to their ecological and functional guilds based on the previous classification by Henriques et al. (2008) and updated with available literature and FishBase online database (Froese & Pauly 2012) (Supplementary data II). Species were considered invertebrate feeders when they feed mostly on non-planktonic invertebrates, otherwise being considered zooplanktivore. Macrocarnivores feed both on

macroinvertebrates and vertebrates (mostly fish). Herbivores feed predominantly on macroalgae, macrophytes, phytoplankton and microphytobenthos and omnivores feed on detritus, filamentous algae, macrophytes, epifauna and infauna. The concepts of habitat association were adapted from Fasola et al. (1997) and the species that use all, or most, habitat categories, especially rocky habitats and less water column and sand, were considered generalists. Finally, the commercial value of each species was attributed based on Cabral et al. (2008).

With the purpose of characterizing structural and functional changes due to fishing, the following metrics were calculated: density of invertebrate feeders, density of omnivores, density of macrocarnivores, density of herbivores, density of generalist individuals, density of juveniles, density of individuals with high commercial value, density of large individuals with medium to high commercial value (> 20 cm) and density of adults with high commercial value (see Henriques et al. 2013 for metrics description). These metrics were selected based on the results of a previous study that analysed the response of several fish-based metrics to different types of human pressure, including fishing (Henriques et al. 2013), and complemented with other metrics that comprise the density and size of target species, as these species are likely to respond quickly to fishing closure (Halpern & Warner 2002). Moreover, the cryptobenthic species belonging to the families Gobiidae, Bleniidae, Gobiesocidade and Tripterygiidae were excluded from the analysis in order to minimize the potential influence of microhabitat, since they depend directly on substratum type (Fasola et al. 1997) and it would not be expected that they benefit strongly from reserve protection (Mosqueira et al. 2000).

Data analysis

Multivariate analysis of variance using permutations (PERMANOVA) tests the effect of one or more factors on one or more variables on the basis of any distance or dissimilarity measure of choice and does not assume normality of errors since the p-values are obtained by permutations (Anderson et al. 2008). Nevertheless, PERMANOVA is sensitive to differences in dispersion among groups, and therefore homogeneity of multivariate dispersions was tested using a PERMDISP routine before running the PERMANOVA tests (Anderson et al. 2008).

The similarity of habitat complexity among zones was tested using two-way PERMANOVA analyses for biotic cover (functional groups of algae and presence/absence of invertebrate groups) and one-way PERMANOVA analysis for habitat structure (CTI and the percent

cover of rock, cobble and sand). When significant values of biotic cover were found for factor zone, univariate PERMANOVA analyses were performed on each variable individually, in order to find those responsible for the differences. The effects of different protection levels and seasonality on fish-based metrics were analysed both through a multivariate (all metrics) and a univariate (each metric individually) perspective using 2-way PERMANOVA analyses (Anderson et al. 2008). With the exception of habitat structure, all analyses were performed with both factors zone (3 levels) and season (4 levels) treated as fixed. Only the factor zone was tested for habitat structure since it is not expected to change seasonally. When significant differences were detected, factors were investigated through *post-hoc* pair-wise comparisons.

In order to visualize multivariate patterns of fish-based metrics without constraints, Principal Coordinates Analysis was used (PCO; Anderson et al. 2008). In addition, Canonical Analysis of Principal Coordinates (CAP; Anderson & Willis 2003) was also performed with the purpose of uncovering patterns that could be masked by unconstrained analysis, by finding axes through the multivariate cloud that best discriminate between different zones and seasons. Furthermore, Spearman correlation coefficients of metric values with PCO and CAP axes were calculated and the most correlated metrics ($r > |0.5|$) supported the discussion of the observed patterns.

All the analyses performed with fish-based metrics and habitat structure variables were based on Euclidean distance matrices, constructed after normalizing each variable by subtracting the mean and dividing by the standard deviation, in order to place all variables on a comparable scale. For algae functional groups, the percentage of cover was fourth-root transformed and the Bray-Curtis similarity index used to construct the resemblance matrix, while for the presence/absence of invertebrate groups the resemblance matrix was calculated using the Jaccard index. All the above-mentioned analyses were performed using PRIMER 6 with PERMANOVA+ software package. P-values were calculated using 9999 permutations and the level of statistical significance adopted was 0.05. After running the analyses, redundancy between metrics was checked and all of them were preserved since no pair was found with a Spearman correlation coefficient higher than |0.85|.

Finally, the size structure of the most abundant species with high commercial value (*Diplodus vulgaris* and *Diplodus sargus*) was plotted per zone and season in order to better understand the effects of fishing and seasonal variability. Size structure was plotted according to the following size classes: early juveniles (below 10 cm), juveniles (between 10 cm and the size at first maturity) and adults (above the size at first maturity).

Results

The analysis of habitat variables showed no significant differences for habitat structure among zones (Pseudo-F = 1.96 $p > 0.05$), as well as for both the factor zone and the interaction between factors zone x season in the case of algae cover (Pseudo-F = 2.02 $p > 0.05$ and Pseudo-F = 1.60 $p > 0.05$, respectively). Regarding the presence of invertebrate groups, the overall multivariate PERMANOVA results revealed significant differences for the factors zone and season (Pseudo-F = 5.17 $p < 0.05$ and Pseudo-F = 1.60 $p < 0.05$, respectively) but, more importantly, no significant differences for the interaction of both factors (Pseudo-F = 1.02 $p > 0.05$).

Pair-wise comparisons for the factor zone in the PERMANOVA analyses performed on each invertebrate group individually showed that only the hydrozoans, anemones, gorgonians and ascidians were significantly different among zones (Pseudo-F = 5.94 $p < 0.05$, Pseudo-F = 11.04 $p < 0.05$, Pseudo-F = 10.76 $p < 0.05$ and Pseudo-F = 4.50 $p < 0.05$, respectively). With the exception of gorgonians, which differ in the TPZ when compared to the remaining zones, hydrozoans and anemones differ in the CPZ when compared to the PPZ and the TPZ, while ascidians only differ between the CPZ and the TPZ. Habitat structure (substrate and topography) features are shown in Figure 3.1, while the algae cover and the frequency of occurrence of invertebrates are presented in Table 3.1.

In the present study, a total of 47 fish species belonging to 20 families were counted in the Arrábida MPA. Sparidae and Labridae were the most represented families in terms of number of species (12 and 9 species, respectively) and abundance (95% of the total abundance, on average). PERMANOVA for the fish-based metrics showed a significant overall multivariate effect of both season and zone (Pseudo-F = 7.47 $p < 0.05$ and Pseudo-F = 5.68 $p < 0.05$, respectively) as well a significant interaction effect (Pseudo-F = 1.51 $p < 0.05$). Additionally, no significant differences in multivariate dispersions was found by the PERMDISP routine ($F = 0.23$ $p > 0.05$). Pair-wise comparisons showed significant differences among all zones and seasons ($p < 0.05$), except between winter and spring ($p > 0.05$), while no consistent patterns were found for the interaction between both factors. Actually, significant differences between all zones were obtained in spring and an opposite effect in winter, no differences were detected between the TPZ and the CPZ in summer and between TPZ and the PPZ in autumn (see pair-wise comparisons in Table 3.2).

Table 3.1 Biotic cover of the sampled habitats: frequency of occurrence of invertebrate groups as well as the mean percentage and standard deviation (in parenthesis) of algae cover by structural groups. Seasons: wi - winter; au - autumn; su - summer; sp - spring. Protection zones: TPZ - total; PPZ - partial; CPZ - complementary.

Biological Cover		TPZ				PPZ				CPZ			
		Sp	Su	Au	Wi	Sp	Su	Au	Wi	Sp	Su	Au	Wi
Frequency of ocurrence of invertebrate groups	Sponges	91.6	66.7	91.7	66.7	50.0	41.7	83.3	58.3	75.0	41.7	83.3	91.7
	Anemones	66.7	91.7	91.7	66.7	83.3	91.7	66.7	83.3	41.7	58.3	33.3	41.7
	Hydrozoans	91.7	33.7	41.7	75.0	100	83.3	33.3	16.7	50.0	33.3	41.7	33.3
	Gorgonians	25.0	16.7	33.3	33.3	16.7	0	8.3	0	0	0	0	0
	Polychaetes	100	100	100	100	100	100	100	83.3	100	100	100	100
	Gastropods	41.7	50.0	50.0	41.7	58.3	58.3	33.3	25.0	50.0	33.3	50	33.3
	Bivalves	0	25.0	16.7	8.3	25.0	0	16.7	25.0	8.3	16.7	41.7	0
	Crustaceans	8.3	33.3	25	0	0	16.7	25	16.7	8.3	25.0	33.3	8.3
	Ascidians	33.3	16.7	8.3	16.7	0	8.3	8.3	8.3	0	8.3	0	0
	Star-fish	0	8.3	0	0	8.3	0	8.3	0	0	0	8.3	0
	Sea urchins	0	16.7	33.3	16.7	8.3	8.3	16.7	25.0	25.0	0	50.0	0
	Sea Cucumbers	0	16.7	0	8.3	16.7	8.3	0	0	0	0	0	0
Algae cover by structural groups (%)	Creeping	39.3 (14.8)	48.9 (15.7)	42.5 (6.5)	37.4 (15.9)	28.0 (17.3)	18.9 (24.4)	16.6 (9.3)	22.8 (20.6)	15.1 (6.5)	41.8 (8.5)	18.4 (6.7)	24.5 (11.8)
	Tufts	19.7 (8.4)	17.0 (8.1)	11.6 (4.2)	19.7 (11.8)	18.7 (7.7)	48.1 (29.6)	20.7 (13.8)	13.6 (1.6)	66.2 (1.4)	11.9 (6.5)	19.4 (9.2)	31.6 (7.3)
	Encrusting	14.8 (12.9)	11.2 (9.3)	35.0 (5)	23.9 (15.3)	13.3 (9.3)	9.2 (9.2)	31.8 (21.2)	46.6 (14.8)	11.2 (0.7)	12.1 (4.5)	51.7 (9.8)	30.4 (11.7)
	Filamentous	0.5 (1.2)	3.9 (3.1)	6.5 (3.1)	0.5 (1.2)	0.5 (1.2)	3.1 (2.2)	9.4 (5.1)	0	1.5 (2.2)	2.9 (4.7)	0.3 (0.6)	0.5 (1.2)
	Sheet	0.9 (2.1)	3.9 (4.7)	0.3 (0.6)	0	3.1 (3.1)	3.4 (4.9)	0.2 (0.4)	0.7 (1.2)	0	11.6 (8.5)	2.7 (2.9)	1.4 (3.3)

Table 3.2 P-values of pair-wise comparisons using permutations of the t-statistic for the interaction between factor zone and season. TPZ - total protection zone, PPZ - partial protection zone and CPZ - complementary protection zone.

	Spring	Summer	Autumn	Winter
TPZ vs. PPZ	0.005*	0.009*	0.278	0.292
TPZ vs. CPZ	0.013*	0.700	0.003*	0.482
PPZ vs. CPZ	0.031*	0.008*	0.003*	0.552

* p-value < 0.05.

The PCO plots show a strong effect of season when compared to the effect of zone, since all seasons, particularly autumn, are clearly separated in the multivariate data cloud. No patterns of zones were globally detected if seasons were not taken into account (Figures 3.2A and 3.2B). The discriminant CAP analysis, however, was able to find axes to separate zones (Figure 3.2C), with a squared canonical correlation of $\delta^2 = 0.523$ ($p < 0.05$).

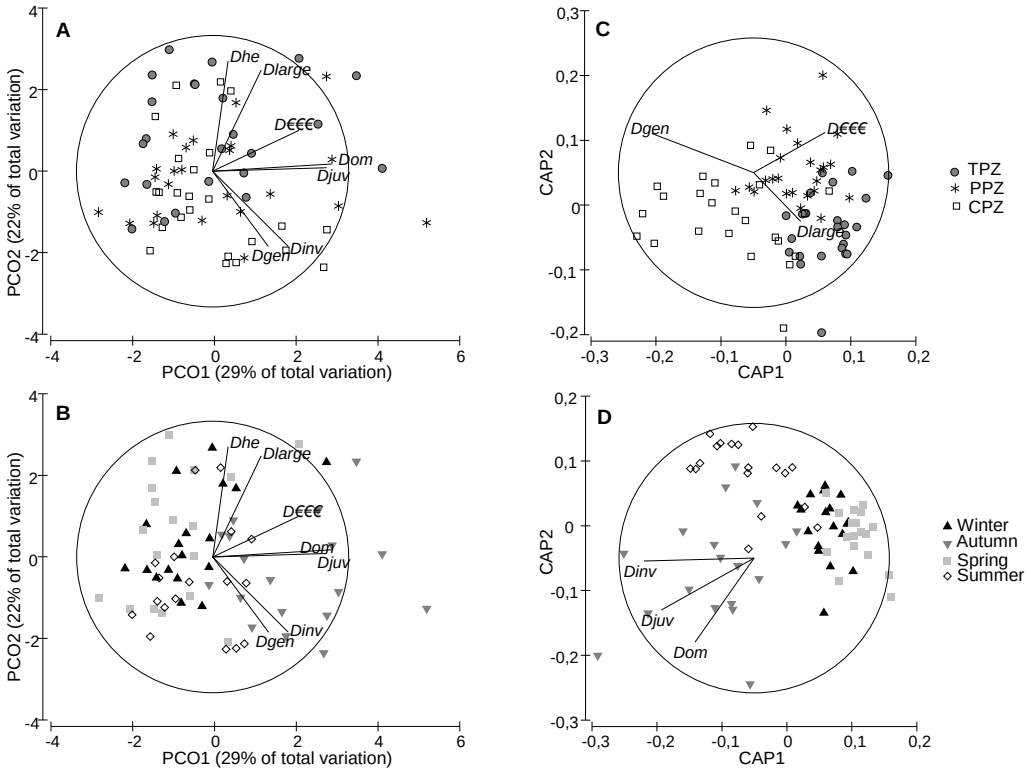

Figure 3.2 Ordination plots of Principal Coordinates Analysis (PCO) and Canonical Analysis of Principal Coordinates (CAP) comparing fish-based metrics among levels of protection (A and C) and seasons (B and D). Correlations with canonical axes are only shown when Spearman's r > |0.5| (circles represent vector correlations of 1). Metric codes: Dinv - density of invertebrate feeders; Dom - density of omnivores; Dgen - density of generalist individuals; Djuv - density of juveniles; D€€€ - density of individuals with high commercial value; Dlarge - density of large individuals with medium to high commercial value (>20cm).

The first canonical axis clearly separated the fish-based metrics in the CPZ from the TPZ and PPZ, and the second canonical axis separated the TPZ from the PPZ (Figure 2C). Vectors representing Spearman correlations with CAP axes ($r > |0.5|$) showed that some metrics are apparently associated with the different levels of protection. The density of generalist individuals is higher in zones where fishing is permitted (CPZ), the individuals with high commercial value tends to have higher values in protected zones (TPZ and PPZ) and the large individuals with medium to high commercial value is associated with the TPZ. The CAP analysis performed to discriminate seasons, as expected, was able to find axes that maximize seasonal variation with a square canonical correlation of $\delta^2 = 0.743$ ($p<0.05$) (Figure 3.2D).The first canonical axis clearly separated the fish-based metrics in autumn and summer from spring and winter, with winter and spring seeming to cluster. In this case, the density of invertebrate feeders was associated with summer and autumn, while the juveniles and omnivores seems to be more related with autumn ($r > |0.5|$) (Figure 3.2D).

Permutational univariate ANOVA on fish-based metrics revealed a significant effect of season in the density of omnivores and juveniles, while significant differences were found among zones for herbivores and the individuals with high commercial value (Table 3.3). Both factors had significant effects on the density of generalist individuals, invertebrate feeders and adults with high commercial value, while no effects were obtained for the macrocarnivores and the large individuals with medium to high commercial value (Table 3.3). Additionally, the interaction between factors zone and season showed a significant effect in the density of invertebrate feeders, density of generalist individuals and density of individuals with high commercial value (Table 3.3). Pair-wise comparisons of metrics with significant results for factor season showed that, in general, the majority of them were significantly different between autumn and other seasons (Table 3.3). Furthermore, some metrics were not significantly different between successive seasons as well as between summer and winter (Table 3.3). Regarding the effects of zone, only the density of generalists showed significant differences among all zones, while the density of invertebrate feeders and the density of individuals with high commercial value differed significantly between the CPZ and the zones with higher protection status (TPZ and PPZ) (Table 3.3). However, this effect of zone was not consistent among all seasons (see pair-wise comparisons for the interaction of both factors in Table 3.3). The density of generalist individuals was consistently different among all zones in all seasons with exception of summer. For the remaining fish-based metrics, no differences between protection zones was detected in winter. In spring, the density of generalists was significantly different between the TPZ and the CPZ.

Table 3.3 PERMANOVA results for the effects of seasons, zones and their interaction on each metric individually (*p-values < 0.05). Shaded areas denote significant results for pair-wise tests (p-values < 0.05). Seasons: wi -winter; au - autumn; su - summer; sp - spring. Protection zones: TPZ - total; PPZ - partial; CPZ - complementary.

Fish-based Metrics	Permutational ANOVA results			Pair-wise comparisons for metrics with significant results for factors									Pair-wise comparisons for metrics with significant results for the interaction between factors											
				Season						Zone			Spring			Summer			Autumn			Winter		
	Factor	Pseudo-F	P(perm)	Wi vs. Au	Wi vs. Sp	Wi vs. Su	Au vs. Sp	Au vs. Su	Sp vs. Su	TPZ vs. PPZ	TPZ vs. CPZ	PPZ vs. CPZ	TPZ vs. PPZ	TPZ vs. CPZ	PPZ vs. CPZ	TPZ vs. PPZ	TPZ vs. CPZ	PPZ vs. CPZ	TPZ vs. PPZ	TPZ vs. CPZ	PPZ vs. CPZ	TPZ vs. PPZ	TPZ vs. CPZ	PPZ vs. CPZ
Density of invertebrate feeders	Season	38.899	0.0002*																				
	Zone	13.163	0.0002*																				
	SexZo	3.2860	0.0054*																				
Density of omnivores	Season	21.164	0.0002*																				
	Zone	2.0490	0.1344																				
	SexZo	0.9660	0.4636																				
Density of macrocarnivores	Season	0.6557	0.7066																				
	Zone	1.9109	0.1140																				
	SexZo	1.1162	0.3370																				
Density of herbivores	Season	1.9197	0.1280																				
	Zone	5.1529	0.0068*																				
	SexZo	1.3598	0.2428																				
Density of juveniles	Season	25.608	0.0002*																				
	Zone	0.0049	0.9514																				
	SexZo	0.7969	0.5834																				
Density of large individuals with medium to high commercial value	Season	1.5915	0.1934																				
	Zone	2.5446	0.0860*																				
	SexZo	1.0556	0.3970																				
Density of generalist individuals	Season	4.1374	0.0104*																				
	Zone	29.133	0.0002*																				
	SexZo	3.1985	0.0090*																				
Density of adults with high commercial value	Season	3.1698	0.0300*																				
	Zone	3.4933	0.0420*																				
	SexZo	1.0013	0.4358																				
Density of individuals with high commercial value	Season	1.8251	0.1514																				
	Zone	5.9448	0.0046*																				
	SexZo	2.3870	0.0418*																				

On the other hand, in autumn, all metrics were different between the CPZ and the remaining zones and no differences in the density of individuals with high commercial value and invertebrate feeders were detected between the TPZ and the PPZ. Finally, plots with size distribution of the most representative species with high commercial value (*D. vulgaris* and *D. sargus*) showed differences among seasons (Figure 3.3). Abundance of juveniles of *D. vulgaris* peaked in summer and no strong peak was identified for early juveniles of *D. sargus*. In general, mean number of adults of *D. vulgaris* was higher in autumn and when higher differences between protection zones were observed, especially between the TPZ and the PPZ in comparison with the CPZ. In the case of *D. sargus*, the mean number of juveniles and adults was higher in winter and spring and the juveniles class was the most represented in all seasons.

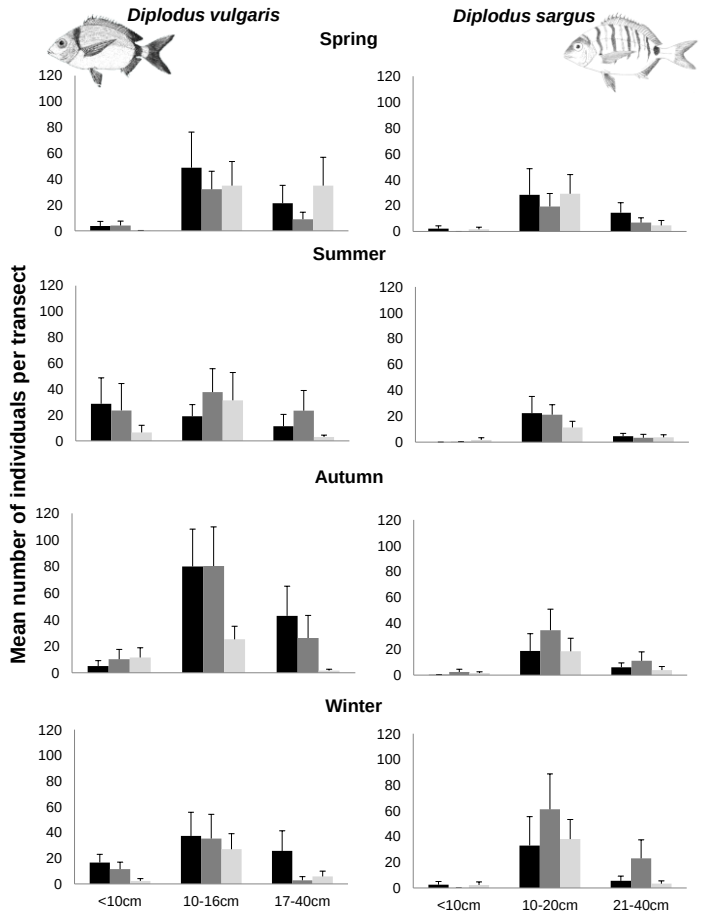

Figure 3.3 Size structure of the most abundant species with high commercial value (*Diplodus vulgaris* and *D. sargus*) per season. Vertical axes correspond to the mean number of individuals per transect in each level of protection: black - total, dark grey - partial and light grey - complementary. Vertical error bars represent standard errors. Size classes: early juveniles (below 10cm), juveniles (between 10 cm and the size at first maturity) and adults (above the size at first maturity).

Discussion

The present study highlights the influence of seasonal variability in fish assemblage patterns and stresses the importance of taking seasonality into account when assessing changes due to anthropogenic disturbance. A marked degree of seasonal variability of the fish-based metrics was evident in both PCO and CAP analyses, which was less pronounced between winter and spring and more in the autumn. These trends were consistent with PERMANOVA results, where significant differences were obtained among all seasons except between winter and spring, matching the results from previous studies that analysed seasonal patterns of rocky fish species (e.g. Holbrook et al. 1994; Friedlander & Parrish 1998; Magill & Sayer 2002; Beldade et al. 2006). Seasonal changes in fish assemblage composition and abundance have been broadly attributed to the input of recruits and to spawning and feeding migrations (Holbrook et al. 1994; Friedlander & Parrish 1998; Aburto-Oropeza & Balart 2001). Indeed, a general increasing trend in the density of juveniles, the omnivores and the invertebrate feeders was associated with summer and autumn samples.

In a study about the very-near-shore fish larvae distribution at Arrábida MPA, Borges et al. (2007) obtained higher diversity and abundance of larvae from May to July followed by a strong decrease in August. This period corresponds to the spawning season of most of the rock-associated species in this area (Almada et al. 1999; Gonçalves et al. 2002; Borges et al. 2007). These results suggest that in this area recruitment processes can occur in late summer. This pattern is also confirmed by Garcia-Rubies and Macpherson (1995) which observed an increase of recruits (smaller than 1.5 cm length) from July to September with a very similar fish assemblage in the NW Mediterranean, although some variances in the starting and range of recruitment pattern of certain species could occur due to temperature differences between Atlantic and Mediterranean waters. It is important to note that the metric density of juveniles includes all individuals bellow the size at first maturity and not merely early juveniles. Consequently, despite the density of juveniles increasing in summer (July), the highest densities were found in autumn (October) as a cumulative result of recruitment processes of various species.

Although the increase in juveniles could partly explain the increased values for the trophic structure metrics that they represent (e.g. omnivores and invertebrate feeders), other factors like spawning migrations or feeding activity might be important (e.g. Harmelin et al. 1995; Friedlander & Parrish 1998).

In temperate rocky reefs, two main spawning strategies occur: (1) demersal spawners, species producing eggs that become attached to the substrate or in nests, normally with males or females displaying parental care, for example the genus Symphodus; and (2) pelagic spawners, species producing planktonic eggs with high dispersive capabilities, for example the families Sparidae, Serranidae and Mugilidae (Almada et al. 1999; Borges et al. 2007). In both cases, females and/or males must travel long distances in search for nests/partners (demersal spawners) or migrate to spawning sites like bays, deep or shallow waters (pelagic spawners) (Almada et al. 1999). These migrations could lead to changes on adult's abundance in the assemblages, either by their departure from reefs or by their aggregation, which is expected to happen at higher intensity in the spawning season from mid-spring to early summer (May-July). In agreement, despite not being initially included in the metrics list, since it had previously shown weak response to anthropogenic pressures (Henriques et al. 2013), the density of individuals over the size at first maturity showed strong seasonal variation (PERMANOVA, Pseudo-F = 4.307 $p < 0.05$ for factor season, Pseudo-F = 1.43 $p>0.05$ for factor zone and Pseudo-F = 1.28 $p > 0.05$ for the interaction of both factors), with significant differences found between summer (July) and spring and autumn (April and October, respectively). Notwithstanding the above-mentioned results, some species exhibited an extended spawning strategy with several peaks year-long, in order to reduce inter-specific competition as they share similar habitat requirements with other species (Garcia-Rubies & Macpherson 1995). Thus, other seasonal differences in spawning and recruitment patterns could be observed for some species as they have several recruitment and adult migration peaks year-long (e.g. *Diplodus* spp.).

Finally, seasonal changes on feeding activity could also contribute for the observed patterns during summer and autumn, specially the density of invertebrate feeders and density of omnivores. Benthic invertebrates, which are known to increase with proliferation of macroalgae canopy (e.g. fleshy erect algae) during spring and summer (Sala 1997), constitute the prevailing preys of most of the observed fish species (Almada et al. 1999). In accordance, the frequent occurrence of several invertebrate groups (e.g. gastropods, crustaceans and bivalves) started in spring and persisted through autumn, which could be related with seasonal fluctuations of macroalgae cover that showed an increase in algae tufts during spring and summer, as observed by Rubal et al. (2011) in tidepool macroalgal assemblages (north of Portugal). An increment of prey availability during summer and autumn might attract invertebrate feeders and omnivores that forage on rocky substrates, being easier to observe them. However, because these results were based on presence/absence data of major invertebrate groups (frequencies of occurrence), further

research about invertebrate's abundance at lower taxonomic levels is desirable to fully understand seasonal changes in fish assemblages. This could prove to be particularly important in the assessment of anthropogenic pressures that also affect invertebrate assemblages.

Despite the fact that some year-to-year variation on the global values of species abundance is expected due to inter-annual biological and environmental conditions (e.g. oceanographic features, recruitment patterns, larval mortality), coastal fish assemblages display a relatively stable and predictable response to seasonality for both juveniles and adults, with higher abundance in summer and autumn (Holbrook et al. 1994; Harmelin et al. 1995; Magill & Sayer 2002; Beldade et al. 2006) and peak larval density in spring and early summer (Borges 2006).

It is expected that environmental conditions on each season affect all zones in a similar manner. Therefore, and taking into account the similarity between habitats, if differences in fish-based metrics were detected among zones within seasons they are likely to be due to the effects of protection. In fact, non-significant differences were found among zones for the habitat structure and algae cover as well as in the interaction zone x season of biotic cover (algae and invertebrates). Only the sessile ascidians and gorgonians (higher in TPZ) and the hydrozoans and anemones (lower in CPZ) showed significant differences among zones. However, it is unlikely that these differences are responsible for the differences found for fish-based metrics, considering that most of the observed fish species that feed on invertebrates exhibit some flexibility in their diets, with a preference for molluscs (gastropods and bivalves), crustaceans (isopods, amphipods and decapods) and polychaetes, which did not differ among zones (see Stergiou & Karpouzi 2002 for feeding habits details). Moreover, there are two additional facts that also suggest the lack of response of fish assemblages to these particular invertebrate groups: (1) the metric density of invertebrate feeders had higher values outside the reserve (CPZ), where a lower frequency of occurrence of those sessile invertebrates was observed; (2) the interaction of both factors (season and zone) was non-significant for these sessile invertebrates due to large differences between samples, while for the fish-based metrics that responded to protection, significant differences were found.

Several differences among zones were found in spring, summer and autumn and no differences were found in winter, suggesting that seasonal variability of fish assemblages affects the detection of their response to anthropogenic pressures. Many of the fish species undergo regular migrations during the winter to find shelter or to spawn (e.g. deeper water), probably as a response to unfavourable conditions (e.g. thermoregulation,

food availability) which create a general pattern of low abundance in this season (Friedlander & Parrish 1998; Magill & Sayer 2002). These regular movements might clarify why no differences were detected among zones during winter in the multivariate approach as well as for the fish-based metrics individually, with the exception of the density of generalists.

The density of generalists was always higher at the zones with the lowest level of protection in all seasons except in summer, where no effects were found. The most abundant generalist *Coris julis* has a wide habitat range and depth distribution, flexibility of diet and has no commercial value in this area (Harmelin et al. 1995; Henriques et al. 2013). Such flexibility is an advantage in a context of inter-specific competition that is expected to be higher inside protected zones (Mosqueira et al. 2000; Ojeda-Martinez et al. 2007). *Coris julis* may migrate to shelf waters to spawn during the spring and summer (see results of Borges et al. 2007). Although the density of generalists was varied with fishing pressure, some care is needed in their widespread use because in some geographical areas *C. julis* has commercial interest (e.g. Mediterranean Sea), prefers deeper habitats (García-Charton & Pérez-Ruzafa 2001) and there may be other generalist species with higher commercial interest (e.g. *Dicentrarchus labrax, Sparus aurata*). Furthermore, *C. julis* is also tolerant to contamination (Fasulo et al. 2010). In this context, the concept of opportunistic species seems to be more adequate and the metric should therefore be changed to density of opportunistic individuals in order to avoid misinterpretations.

Regarding the trophic structure metrics, no pattern of response to the interaction between the level of protection and season was found for the density of invertebrate feeders even though some significant differences were obtained in pair-wise comparisons. In this case, the most probable explanation is related to the functional overlap between species that responded positively or negatively to protection (*Coris julis* vs. other invertebrate feeders). As for the density of herbivores (only with differences between TPZ and PPZ but with no significant results for the interaction between factors), the most probable explanation is related to the migration of schools between feeding grounds as it only comprises the species *Sarpa salpa*, which has a broad home range (Jadot et al. 2006; Abecasis et al. 2012). These results suggest that these trophic levels were not good indicators of fishing pressure as opposed to macrocarnivores and piscivores (top predators) as the most sensitive groups (e.g. Micheli et al. 2004; Guidetti & Sala 2007; Villamor & Becerro 2012). The low density values observed for top predators could be related not only to the early age of the reserve and their high mobility but also to their preference by other types of habitats (e.g. *Pagrus pagrus*; García-Charton & Pérez-Ruzafa 2001).

Concerning the distribution of fishing activities along the MPA (see material and methods), greater differences would be expected between the CPZ and the remaining zones, since no fishing activity is allowed in the TPZ while in the PPZ only traps, jigs and handlines are allowed farther than 200 m from shore line. This pattern was only observed in the autumn and for the metrics density of generalists and density of invertebrate feeders (as explained before), as well as for the density of individuals with high commercial value. In accordance with the expected pattern, results of CAP showed that the density of large individuals with medium to high commercial value (> 20 cm) was also associated with the TPZ and the PPZ, however, no significant differences were found, meaning that it was not consistently different. On the other hand, the density of individuals with high commercial value was only significantly different between zones in autumn, following the expected pattern due to fishing effort distribution, which is probably due to a cumulative result of the abundance of juveniles and adults during this season, in accordance with the general seasonal pattern observed. These results suggest that this reserve is still in a trajectory of recovery.

An increase of large-bodied species, especially those targeted by fishing, has been pointed out as one of the main changes in fish assemblages due to protection (e.g. Halpern & Warner 2002; Micheli et al. 2004; Claudet et al. 2006; Guidetti & Sala 2007; Claudet et al. 2010). Because large-bodied species are many times slow-growing and late-maturing, they will respond slower to protection measures than short-lived and fast-growing species (Mosqueira et al. 2000; Halpern & Warner 2002). Moreover, the increase in species abundance is extremely dependent on recruitment processes, fishing effort outside and between the different levels of protection, as well as their catchability and migration patterns (Côté et al. 2001; Micheli et al. 2004; Guidetti & Sala 2007). This means that recovery of fish size and species density depends on the age and size of the reserve (Claudet et al. 2008). For instance, some studies suggest that 1−3 yr appears to be enough to detect significant changes in total density and biomass (see meta-analysis of Halpern & Warner 2002), while this time period seems to be insufficient to detect differences in fish size or trophic structure (e.g. the abundance of top predators) (e.g. Micheli et al. 2004; Russ & Alcala 2004; Claudet et al. 2006). In this context, despite the sampled site being under full protection measures for 2 years, there was probably not enough time to detect clear changes on fish size, explaining why no consistent effects of zone were observed for the density of adults with high commercial value and the large individuals with medium to high commercial value (> 20 cm).

A previous study by Harmelin et al. (1995) showed higher abundances of *Diplodus* spp. (especially large individuals) within protected zones in all seasons and successive years,

though these abundances peaked in late summer (Mediterranean Sea). Therefore, the fact that the density of individuals with high commercial value only responded to the effect of zone in autumn and the lack of response of the density of adults with high commercial value, stress the idea that the MPA is at an early stage. In fact, seasonal variations on size structure of the most abundant species with high commercial value, *D. sargus* and *D. vulgaris* were observed. These variations followed the life-cycle features of each species and resulted in clear differences between zones in autumn, probably due to cumulative abundances of both juveniles and adults. Both species have a large spawning period that peaks in winter and results in an extended recruitment (Garcia-Rubies & Macpherson 1995), but while *D. vulgaris* travels to shelf waters (Correia et al. 2011), *D. sargus* congregates in sheltered bays and nearshore waters to spawn (Almada et al. 1999; Veiga et al. 2010). These results are also supported by the research of Horta e Costa et al. (in press) performed in the same study area, during the spring and autumn (pooled together) in two consecutive years (2009-2010), where eight zones were sampled inside TPZ and PPZ (four in each one) and four zones in CPZ (spatial variability). The authors found significant differences in the density of both *D. vulgaris* (below the legal size of 15 cm) and *D. sargus* (all sizes) between protected (TPZ and PPZ) and unprotected zones (CPZ) (Horta e Costa et al. in press).

It is important to note that this study was not based on before-after MPA establishment data, so other factors beyond the ones discussed could hinder the detection of significant differences among the different levels of protection: (1) size of the no-take and partially protected areas relative to the target species' home-range; (2) the ban of spearfishing in the whole MPA area which has been reported to heavily impact target species (e.g. *Diplodus* spp.) (Coll et al. 2004; Lloret et al. 2008; La Mesa et al. 2011b); (3) the increased surveillance of illegal fishing activity. In this way, further research is need to follow the reserve effects over time, like changes on size-structure of large-bodied fish (e.g. target species) and increase in the abundance of top predators (macrocarnivores and piscivores). This should also be complemented with the study of complex successional trajectories of inter-species relationships (e.g. decreases in the abundance of preys due to the increase in top predators) in order to test new metrics. Further studies should include other habitats and depth strata in order to accomplish higher spatial variability.

In conclusion, this study showed that (1) seasonal variability affects the structural and functional features of fish assemblages that can influence the detection of changes as a result of anthropogenic pressures; (2) comparisons between control and impacted sites should only be made using data from similar seasons; (3) it is important to take the species

life history and their ecological traits into account to analyze fish-based metrics, especially those represented by one or few species; (4) the metrics density of generalists (i.e. density of opportunistic individuals) and density of individuals with high commercial value seem to be adequate to assess fish assemblages' response to fishing pressure. Overall, the choice of a particular season to assess impacts on fish assemblages, during warm sea conditions after the spawning period (July-November), can probably give better results while minimizing monitoring costs. This is particularly important in wide-ranging environmental assessments, those required to implement the Marine Strategy Framework Directive.

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Supplementary data II. Database used to calculate fish-based metrics. The list presents the ecological features of each species: Length at first maturity, feeding guilds (inv - invertebrate feeders, ma - macrocarnivores, pi - piscivores, om - omnivores, zoo - zooplanctonivores, he - herbivores), Commercial value (€ - none or low, €€ - medium, €€€ - high), Habitat Association (rockcave - rocky substrate and cavities, watcave - water column and cavities, gen - generalists, rockspe and sandspe - rock and sand substrate specialists, respectively, watalgae - substrate covered by algae and water column, wat - water column).

Species	Family	L maturity	Trophic guild	Commercial Value	Habitat association
Atherina presbyter	Atherinidae	9.7	ma	€	wat
Boops boops	Sparidae	19.8	om	€€	watalgae
Bothus podas	Bothidae	26.5	ma	€€	sandspe
Callionymus lyra	Callionymidae	15	inv	€	sandspe
Callionymus reticulatus	Callionymidae	7.6	inv	€	sandspe
Centrolabrus exoletus	Labridae	8.5	inv	€	watcave
Chelon labrosus	Mugilidae	38.1	om	€€	wat
Chromis chromis	Pomacentridae	9.2	zoo	€	rockcave
Conger conger	Congridae	125.3	ma	€€€	rockcave
Coris julis	Labridae	16.2	inv	€	gen
Ctenolabrus rupestris	Labridae	10.1	inv	€	watcave
Dicentrarchus labrax	Moronidae	44.4	ma	€€€	gen
Diplodus annularis	Sparidae	16	om	€€€	watalgae
Diplodus bellottii	Sparidae	15.6	om	€€€	watalgae
Diplodus cervinus	Sparidae	31.6	om	€€€	watalgae
Diplodus puntazzo	Sparidae	34.1	om	€€€	watalgae
Diplodus sargus	Sparidae	21	om	€€€	watalgae
Diplodus vulgaris	Sparidae	16.5	om	€€€	watalgae
Gaidropsarus mediterraneus	Lotidae	29	om	€	rockcave
Labrus bergylta	Labridae	31.9	inv	€€	watalgae
Labrus mixtus	Labridae	19.9	inv	€€	watcave
Liza aurata	Mugilidae	37.3	om	€€	wat
Mugil cephalus	Mugilidae	54.6	ma	€€	wat
Mullus barbatus	Mullidae	15.5	inv	€€€	sandspe
Mullus surmuletus	Mullidae	26.2	inv	€€€	sandspe
Muraena helena	Muraenidae	76.7	ma	€€€	rockcave
Oblada melanura	Sparidae	20.6	om	€€	watalgae
Pagellus acarne	Sparidae	17.7	ma	€€€	gen
Pagellus bogaraveo	Sparidae	33.6	ma	€€€	gen
Pagrus pagrus	Sparidae	35.2	ma	€€€	gen
Phycis phycis	Phycidae	35.6	inv	€€€	rockcave
Pollachius pollachius	Gadidae	67.5	inv	€€€	gen
Pomatoschistus pictus	Gobiidae	4.5	inv	€	sandspe
Sarpa salpa	Sparidae	28	he	€€	watalgae
Scomber colias	Scombridae	27.8	ma	€€	wat
Scorpaena notata	Scorpaenidae	15.2	ma	€	rockcave
Scorpaena porcus	Scorpaenidae	16.4	ma	€€	rockcave
Serranus cabrilla	Serranidae	13.6	ma	€	watcave
Sparus aurata	Sparidae	31.9	om	€€€	gen
Spondyliosoma cantharus	Sparidae	31.1	om	€€	watalgae
Symphodus bailloni	Labridae	12.9	inv	€	rockspe
Symphodus melops	Labridae	13.1	inv	€	rockspe
Symphodus roissali	Labridae	10.4	inv	€	rockspe
Symphodus rostratus	Labridae	7	inv	€	rockspe
Syngnathus acus	Syngnathidae	29	inv	€	rockspe
Zeugopterus punctatus	Scophthalmidae	15.7	ma	€€	rockcave

CHAPTER 4

Henriques S., Pais M.P., Costa M.J. & Cabral, H.N. Structural and functional changes in a soft-substrate fish assemblage induced by a sewage outfall. In review in Environmental Monitoring and Assessment.

Structural and functional changes in a soft-substrate fish assemblage induced by a submarine sewage outfall

Abstract: Understanding how sewage affects fish assemblages at structural and functional levels is key to predict its effects on ecosystem functioning. This study constitutes the first guild approach regarding the effects of sewage on soft-substrate fish assemblages, by analysing the response of several fish-based metrics to a sewage gradient. The combined results of PERMANOVA and discriminant analyses showed that the metrics related with individuals with low and very low resilience, rock residents and omnivores, had greater sensitivity to sewage pollution, which was attributed to the balance between habitat complexity and effluent effects (toxicity vs. available resources). Moreover, density-based metrics may be better indicators of sewage pollution than biomass-based metrics, since they better reflect changes in the relative abundance of common small-bodied species. Although further research is needed to strengthen the sensitivity and to assess the applicability of the selected metrics, this study improves the understanding of coastal fish assemblage's response to sewage pollution, contributing for their use as biological indicators of human pressures and to the future development of environmental assessment tools, such as multimetric indices.

Keywords: Sewage outfall, demersal fish assemblages, fish-based metrics, soft-substrate habitats, Marine Strategy Framework Directive.

Introduction

Industrial and urban growth and their establishment on the coastlines increased the impacts on surrounding marine communities (Halpern et al. 2008; Crain et al. 2009). Sewage outfalls are one of the main pollution sources in nearshore coastal waters, increasing the levels of pathogenic organisms, organic substances, heavy metals, trace elements, among others (Jordão et al. 2002; Islam & Tanaka 2004; de-la-Ossa-Carretero et al. 2012). The impact caused by a sewage effluent will depend in part on flow rate, depth and hydrodynamic conditions of release and type of wastewater discharged (i.e. domestic or industrial) (Azzurro et al. 2010 and references therein). In recent years, the use of fish assemblages as indicators of anthropogenic disturbance has received an increasing attention due to their relative easiness of identification, representation of several functional guilds that reflect all components of the ecosystem and trophic levels, their rapid response to pulse impacts and a high economic value that facilitates the understanding of environmental degradation by the general public (Whitfield & Elliott 2002; Guidetti et al. 2003).

Although sewage effects on fish assemblages have been studied in rocky and coral reefs (e.g. Pastorok & Bilyard 1985; Grigg 1994; Smith et al. 1999; Guidetti et al. 2002; Guidetti et al. 2003; Fabricius et al. 2005; Reopanichkul et al. 2009; Azzurro et al. 2010;

Reopanichkul et al. 2010), estuaries and bays (e.g. Hall et al. 1997; Araujo et al. 2002) and streams (e.g. Porter & Janz 2003), fewer studies have investigated their effects in marine soft-substrate habitats. Despite that, there are some suggestions that sewage induces changes in abundance of some soft-substrate fish species (Russo 1982; Otway 1995; Scanes & Philip 1995; Otway et al. 1996a; Otway et al. 1996b) with consequences in their trophic interactions (Russo 1982; Otway et al. 1996b). However, none of these studies focused on a complete structural and functional guild approach to fish assemblages, i.e. diversity, abundance, trophic structure, mobility, resilience habitat association, nursery function. Understanding how sewage affects fish assemblages at these levels is key to predict its effects on ecosystem functioning as well as to assess the environmental status of marine waters in the scope of European Directives (e.g. Marine Strategy Framework Directive).

Structurally and functionally, marine fish assemblages are strongly related to their surrounding habitat, being more diverse and abundant as habitat complexity increases, as a result of the food regime accessible (balance between prey and competitors), quantity of shelter (degree of exposure to predators) and conditions that maximize their reproductive potential (García-Charton & Pérez-Ruzafa 2001; Pihl & Wennhage 2002; Rice 2005). In the case of soft-substrate habitats, many studies identify depth as the main structural factor of fish assemblages (e.g. Demestre et al. 2000; Sousa et al. 2005; Catalan et al. 2006), being more homogeneous on deeper zones due to changes in water temperature, pressure, light intensity and salinity, which affect species distribution according to ecological needs and physiological tolerances (Rice 2005). Nevertheless, other factors like latitude and sediment type can have an important effect on species distribution (Gaertner et al. 1998; Demestre et al. 2000; Pihl & Wennhage 2002; Prista et al. 2003; Labropoulou & Papaconstantinou 2004). For instance, Prista et al. (2003) suggest that the proximity of rocky shores increases the heterogeneity of the soft-substrate habitats by creating a wider diversity of available niches, supporting higher fish diversity and abundance. In this context, the assessment of changes on fish assemblages should have well-based information about natural variability, as it is the only way to properly predict consequences of anthropogenic activities (Holbrook et al. 1994).

The purpose of this study was to evaluate the effects of sewage on function and structure of soft-substrate fish assemblages, by comparing and selecting the fish-based metrics that best distinguish among assemblages displaced along a gradient of exposure to a sewage discharge. All fish-based metrics were analysed with both density and biomass data in order to identify the type of data that is most suitable to detect sewage-induced changes.

Material and Methods

Study Area

The case-study is the largest submarine sewage outfall in Portugal, located off Cascais (Guia). It receives wastewaters from four municipalities on western Lisbon (Figure 4.1) and operates since 1994, supplying 753 thousand inhabitants (population equivalent) and discharging 150 thousand m^3 per day of urban effluent (Santos et al., 2011) subject to primary treatment with screening and removal of grit and solids, degreasing and primary decantation without addition of reagents. The submarine outfall is 2.75 km long, including two 1.8 km long diffusers, and lies at a depth of 45 m. The last 400 m of the diffusers have 80 holes (every 5 m) to promote the dilution process during the discharge (Neves et al. 2002; Santos et al. 2002; Santos et al. 2011).

The impact caused by the sewage effluent on the physicochemical parameters of water column and sediments was previously detected in a restricted area close to the diffuser, as a consequence of the depth of the discharge zone, strong tidal currents, wind and wave action that promotes the initial dilution and sediment ressuspension (Neves et al. 2002). The results of the monitoring program implemented on this outfall showed that the plume extends most in mid and bottom waters (Santos et al. 2002; Santos et al. 2011). Moreover, in typical conditions the plume disperses northward (see Neves et al. 2002 for details). The plume is only identifiable by faecal bacteria which are present in higher concentrations close to the diffusers (< 2 km), still measureable at ~ 4 km distance (with less intensity), but at ~ 8 km from diffuser they are hardly detectable (Santos et al. 2002).

Accounting for the above-mentioned characteristics of the plume, three zones were selected in order to represent a gradient of sewage influence. Site A was located near the mouth of the outfall while sites B and C were placed 4 and 8 km away from the outfall, respectively (Figure 4.1). These sites were sampled at the end of summer 2010 (September) to avoid the effect of seasonality on fish assemblages and prevent the strong influence of the Tagus estuary plume that has higher flow during wet months (Valente & da Silva 2009).

Fish-based metrics

On board of a fishing vessel, nine 30-minute trawls were performed using an otter-trawl (12 m headline; 20 m footrope; 80 mm cod-end mesh), three replicates randomly allocated within each zone, covering a total area of 147.000 m^2. Trawls were carried out in daylight at a constant speed (2.1-2.3 knots) and all fish were identified, measured (total length; 1

mm precision) and weighted (0.01 g precision). On all sites, trawls were performed within the 47−62 m depth range in order to reduce the influence of depth on fish assemblages (Figure 4.1). GPS location was recorded at the start and end points of each trawl in order to estimate the total sampled area and calculate density (ind.1000 m^2) and biomass (kg.1000 m^2) per species for each replicate.

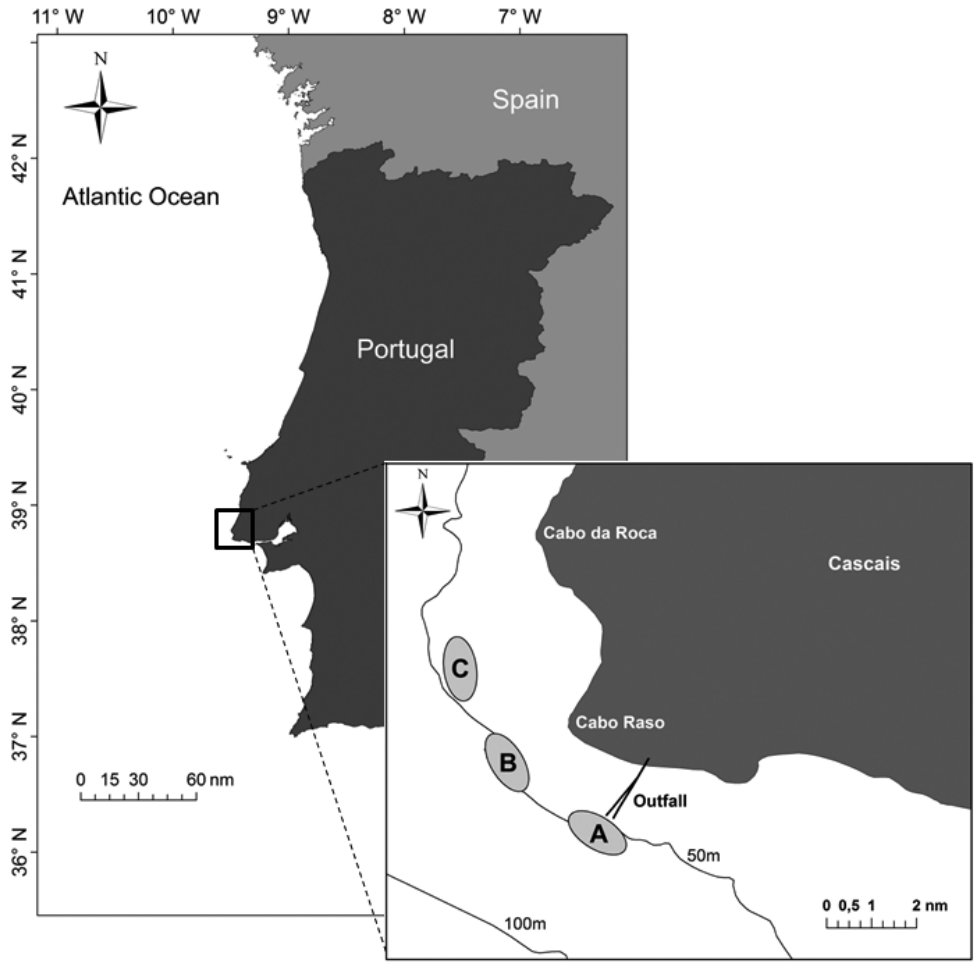

Figure 4.1 Location of the sampled sites that represent the sewage gradient: A - site near the outfall, B and C - sites 4 and 8 km away from the outfall, respectively.

All fish species caught were classified into functional guilds: commercial value, trophic guilds, resilience and dependence or residence in specific habitat type (Supplementary data III). Guild classification and concepts were adapted from Henriques et al. (2008) and

FishBase online database (Froese & Pauly 2012). Trophic guilds were adapted from Elliott et al. (2007).

A total of 28 fish-based metrics were calculated in order to analyse both structural and functional changes due to sewage discharges (Table 4.1). These metrics represent a broad range of fish assemblage attributes such as diversity, abundance, trophic structure, resilience, habitat association, mobility and nursery function, and were selected based on ecological features of soft-substrate fish assemblages and their response to anthropogenic pressures (Russo 1982; Otway 1995; Otway et al. 1996b; Porter & Janz 2003; Rochet & Trenkel 2003; Labropoulou & Papaconstantinou 2004; Henriques et al. 2008; Johnston & Roberts 2009; McKinley & Johnston 2010).

Metrics sensitivity

The identification of the most sensitive metrics was performed by examining general patterns of fish-based metrics on each site along the exposure gradient, through one-way permutational multivariate (all metrics) and univariate (each metric individually) analyses of variance with site treated as fixed factor (PERMANOVA; Anderson et al. 2008) and discriminant analysis through a Canonical Analysis of Principal Coordinates (CAP; Willis & Anderson 2003). *Post-hoc* pair-wise comparisons followed whenever significant differences among sites were found. Spearman correlation coefficients of the fish-based metrics with CAP axes were calculated and the highest values ($r > |0.5|$) were added to the plots in order to support the discussion of the observed patterns.

After running the analyses, all metrics were normalized by subtracting the mean and dividing by the standard deviation to place all metrics on a comparable measurement scale. Analyses were performed based on Euclidean distance matrices using PRIMER 6 with PERMANOVA+ software package. Results were considered significant at $p < 0.05$ and p-values were calculated through 9999 permutations. In PERMANOVA analyses, whenever the number of unique permutations available did not reach 100 due to lack of replicates, p-values were based on a Monte Carlo method (Anderson et al. 2008).

Additionally, Spearman correlations among metrics were calculated to detect redundancies ($r > |0.85|$) in order to proceed to the final choice of the most sensitive metrics. After the exclusion of redundant metrics, a metric was selected as sensitive if it was significantly different among sites and if it had high correlation with CAP axes. This analytical approach provides detailed information about fish assemblage-level indicators to assess functional

impacts of sewage discharges. Spearman correlations were performed in Statistica 10 software.

Finally, the SIMPER routine was used to identify the percent contribution of each species for the average Bray-Curtis dissimilarity among sites (Clarke & Gorley 2006), in order to improve the interpretation of differences in metric values. This analysis was performed using PRIMER 6 software.

All analyses were run with fish-based metrics estimated in density and in biomass with the purpose of identifying which type of data is most sensitive to sewage induced changes.

Results

The sewage outfall studied led to several changes on fish assemblages, with metrics belonging to different attributes responding to sewage pressure. Overall, significant differences were found among sites with both density (PERMANOVA; Pseudo-F = 4.814, $p < 0.05$) and biomass (PERMANOVA; Pseudo-F = 10.502, $p < 0.05$) data. Moreover, pair-wise comparisons showed that when metrics were measured in biomass all sites differed significantly ($p < 0.05$), while with density data the sites B and C were significantly different from the site near the sewage outfall (A) ($p < 0.05$).

In accordance, results of PERMANOVA on each metric individually showed increased differences among sites when biomass data was used, accounting for the larger number of metrics responding significantly to the factor site (22 metrics), which contrasted with the 15 sensitive metrics obtained with density data (Table 4.2). These differences were due to the metrics total density/biomass, density/biomass of flatfish, density/biomass of invertebrate feeders, density/biomass of macrocarnivores, density/biomass of individuals with medium mobility, density/biomass of individuals with high resilience and density/biomass of soft-substrate dependents which only differed between sites when measured in biomass. Moreover, metrics related with juveniles, sedentary, and medium commercial value individuals were not significantly different among sites with none of the data types ($p > 0.05$) (Table 4.2).

In general, the metrics that characterized the replicates nearest the outfall were the density/biomass of omnivores, density/biomass of rock residents and density/biomass of individuals with medium resilience (Table 4.2), always showing higher values on site A.

Table 4.1 List of candidate metrics to assess the response of soft-substrate fish assemblages to sewage discharges. Metrics are divided by the following attributes: Diversity/composition, trophic structure, mobility, resilience, habitat association and nursery function.

Metric	Abbreviation	Description
Species diversity/composition/abundance		
Total number of species	Tspp	Measure of species richness
Total density or biomass (Ind or Kg /1000m2)	Tdens or Tbiom	Measure of abundance
Dominance	DOM	Number of species that make up 90% of the total density or biomass
Mean trophic level	MTL	
Average fish lenght	Av_lenght	Measures of the assemblage structure
Average lenght of adults	Av_adults_lenght	
Density or biomass of Chondrichthyes	Chondrichthyes	Measures of conservation value of the system
Density or biomass of Flatfish	Flatfish	
Density or biomass of individuals with medium commercial value	€€	Measures of commercial threatened
Density or biomass of individuals with high commercial value	€€€	
Trophic structure		
Density or biomass of "invertebrate feeders"	inv	Feed predominantly on non-planktonic invertebrates
Density or biomass of omnivores	om	Feed on detritus, filamentous algae, macrophytes, epifauna and infauna
Density or biomass of macrocarnivores	ma	Feed on macroinvertebrate and vertebrates (mostly fish)
Mobility		
Density or biomass of individuals with high mobility	hm	Moved over relatively large distances
Density or biomass of individuals with medium mobility	mm	Daily movement patterns on the order of tens of meters
Density or biomass of sedentary individuals	se	Limited moviment and well defined home ranges
Resilience		
Density or biomass of individuals with "very low" and "low" resilience	VL+L	Capacity to recover from changes in the environment; Minimum population dubling time: high (up to 1.4 years), medium (1.4 to 4.4 years), low (4.5 to 14 years), very low (more than 14 years) (values available on fishbase)
Density or biomass of individuals with "medium" resilience	M	
Density or biomass of individuals with "high" resilience	H	
Habitat association		
Density or biomass of rocky residents	Rocky_re	Dependency from substrate type; species considered resident used the associated substrate permanently while dependents only used these types of substrate to feed, shelter or spawn
Density or biomass of soft-bottom residents	Soft-b_re	
Density or biomass of rocky dependents	Rocky_dep	
Density or biomass of soft-bottom dependents	Soft-b_dep	
Nursery function		
Density or biomass of adults	adults	Individuals over the maturity size (sizes information collected from fishbase)
Density or biomass of juveniles	juv	Individuals bellow the maturity size

On the other hand, the mean trophic level metric had lower values on this site, Chondrichthyes and individuals with very-low and low resilience were absent from this location and were always present in the replicates from sites B and C. Furthermore, only the metrics mean trophic level and density/biomass of omnivores responded clearly to the expected gradient effect, with values increasing and decreasing, respectively, with the distance from the outfall.

Table 4.2 PERMANOVA results for the effects of sewage (sites) on each metric individually, using both density and biomass data. Shaded areas denote significant results for pair-wise permutational t-tests (p-values < 0.05). Sites: A - near outfall, B - 4 km away from outfall and C - 8km away from outfall.

Fish-based metrics	PERMANOVA results		Pair-wise comparisons					
	p-values		density			biomass		
	density	biomass	A-B	A-C	B-C	A-B	A-C	B-C
Total number of species	0.028							
Total density or biomass (Ind or Kg /1000 m^2)	0.32	0.006						
Dominance	0.004	0.005						
Mean trophic level	0.005							
Average fish lenght	0.01							
Average lenght of adults	0.026							
Density or biomass of Chondrichthyes	0.006	0.002						
Density or biomass of Flatfish	0.095	0.018						
Density or biomass of individuals with medium commercial value	0.458	0.145						
Density or biomass of individuals with high commercial value	0.011	0.004						
Density or biomass of invertebrate feeders	0.063	0.006						
Density or biomass of omnivores	0.004	0.007						
Density or biomass of macrocarnivores	0.576	0.003						
Density or biomass of individuals with high mobility	0.016	0.01						
Density or biomass of individuals with medium mobility	0.573	0.003						
Density or biomass of sedentary individuals	0.465	0.152						
Density or biomass of individuals with very low and low resilience	0.004	0.003						
Density or biomass of individuals with medium resilience	0.004	0.004						
Density or biomass of individuals with high resilience	0.093	0.035						
Density or biomass of rocky residents	0.034	0.011						
Density or biomass of soft-bottom residents	0.408	0.017						
Density or biomass of rocky dependents	0.003	0.009						
Density or biomass of soft-bottom dependents	0.023	0.034						
Density or biomass of adults	0.037	0.003						
Density or biomass of juveniles	0.779	0.419						

These results were also supported by the discriminant analysis performed by CAP, where the first canonical axis clearly separated the fish-based metric values of site A from those of the sites B and C, when measured with both density ($\delta^2 = 0.975$; $p < 0.05$) and biomass ($\delta^2 = 0.973$; $p < 0.05$) data (Figure 4.2). Moreover, the second CAP axis distinguished site B from remaining sites. Vectors representing the Spearman correlations with the CAP axes showed that several metrics were related with this pattern ($r > |0.5|$) (Figure 4.2). Despite

that, the metrics density/biomass of rock residents, density/biomass of sedentary individuals, density/biomass of omnivores, dominance when measured in biomass, density/biomass of rock dependents and density/biomass of individuals with medium resilience seem to be associated with site A, while the metrics mean trophic level, density/biomass of flatfish, density/biomass of Chondrichthyes, density/biomass of individuals with low and very low resilience were linked with sites B and C (Figure 4.2). It is also important to note that site B (4 km away from the outfall) was characterized by higher values of total biomass, thus many of the metrics measured in biomass were associated with the second axis of the CAP plot (Figure 4.2).

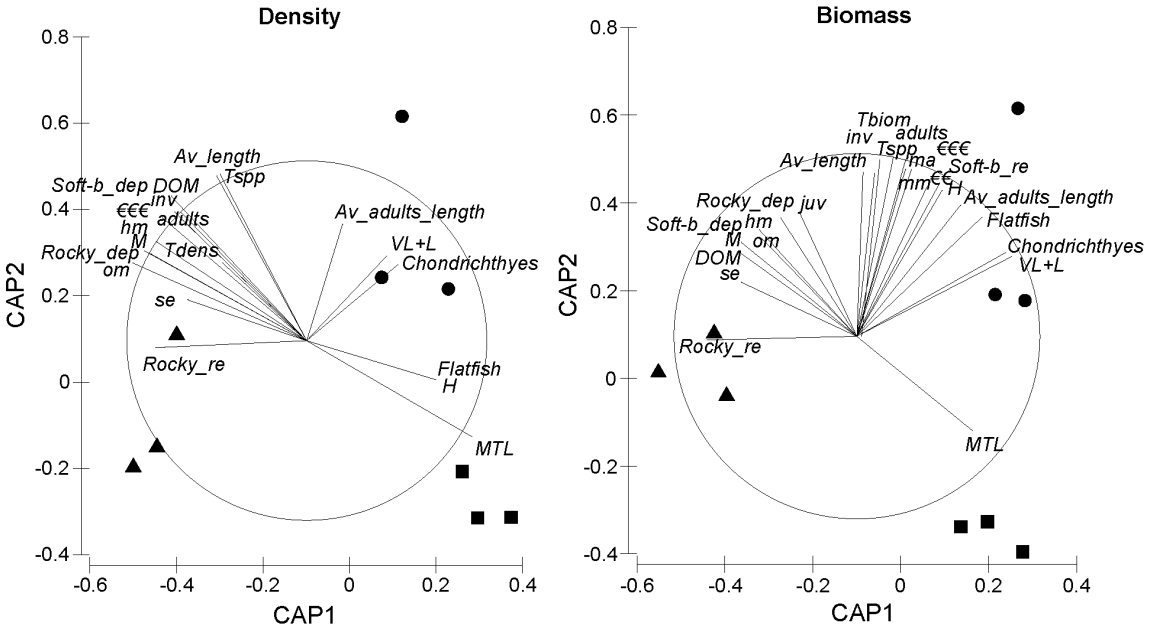

Figure 4.2 Ordination plots of the Canonical Analysis of Principal Coordinates (CAP) comparing fish-based metrics among sites along the gradient of exposure to sewage with both density (A) and biomass (B) data. Triangles represent site A (outfall), while circles and squares represent sites B (4 km away) and C (8 km away), respectively. Correlations with canonical axes are only shown when Spearman's r > |0.5|. For metric abbreviations see Table 4.1.

Overall, none of the metrics belonging to the mobility and nursery function attributes, relative to the length of individuals and commercial value, as well as the metrics total number of species and total density, were useful in distinguishing among sites.

Regarding the metrics with higher sensitivity to the impact of sewage (PERMANOVA and CAP results), Spearman correlations among each pair of metrics showed that the metric density/biomass of Chondrichthyes was redundant with the density/biomass of individuals

with low and very low resilience, as well as the density/biomass of rock dependents, density/biomass of omnivores, density/biomass of individuals with medium resilience among them (r > |0.85|).

Table 4.3 Summary of SIMPER results showing the average density (ind.1000 m^2) and biomass (Kg. 1000 m^2) values among sites (A - near outfall, B - 4 km away from outfall and C - 8 km away from outfall). A cut-off appoint of 90% cumulative dissimilarity was applied.

Data type	Species	A		B		C
Density	*Arnoglossus imperialis*[a]	5.73	<	7.3	<	8.58
	Arnoglossus thori	0.33	>	0	<	0.02
	Aspitrigla cuculus	0.3	<	0.66	>	0.18
	Buglossidium luteum	0.02	<	0.06	<	0.19
	Citharus linguatula	0.35	<	0.55	>	0.38
	Callionymus lyra[a]	3.57	>	3.16	>	2.07
	Chelidonichthys lucernus	0.2	>	0.02	=	0.02
	Chelidonichthys obscurus	0.16	>	0.02	<	0.06
	Citharus linguatula	0.35	<	0.55	>	0.38
	Dicologlossa cuneata	0.15	<	0.49	>	0.42
	Diplodus bellottii[a]	1.22	>	0.31	>	0.02
	Lepidotrigla cavillone	0.2	>	0.12	>	0.06
	Merluccius merluccius	0.07	<	0.15	>	0.1
	Microchirus azevia	0.3	<	0.57	>	0.07
	Raja clavata	0	<	0.59	>	0.35
	Serranus hepatus	0.2	>	0	=	0
	Spondyliosoma cantharus	0.06	<	0.18	>	0.09
	Trisopterus luscus[a]	1.59	>	0.7	>	0.24
Biomass	*Arnoglossus imperialis*	0.05	<	0.08	>	0.07
	Aspitrigla cuculus	0.01	<	0.02	=	0.01
	Callionymus lyra[a]	0.24	>	0.21	>	0.12
	Chelidonichthys lucernus	0.02	>	0	=	0
	Dicologlossa cuneata	0.01	<	0.05	=	0.03
	Diplodus bellottii[a]	0.09	>	0.03	>	0
	Diplodus vulgaris	0.02	>	0	=	0
	Microchirus azevia	0.03	<	0.07	>	0.01
	Raja clavata[a]	0	<	0.64	>	0.11
	Rostroraja alba	0	<	0.09	>	0
	Scyliorhinus canicula	0	<	0.03	>	0
	Solea solea	0.01	<	0.03	>	0.01
	Spondyliosoma cantharus	0.01	<	0.04	>	0.02
	Trisopterus luscus[a]	0.16	>	0.07	>	0.02

[a] Species that contributed to over 10% of the dissimilarities among sites

Moreover, the metric density/biomass of omnivores was negatively correlated with the metric mean trophic level ($r < -0.85$), while the metric density/biomass of rock residents was not redundant with any other metric. Taking these results into account, the metrics density/biomass of individuals with low and very low resilience, density/biomass of rock residents and density/biomass of omnivores were selected as the most suitable to detect changes on soft-substrate fish assemblages due to sewage discharge. The metric density/biomass of omnivores was selected rather than mean trophic level since it was correlated with a higher number of metrics that were not selected.

Finally, the results of the SIMPER analysis indicate that the species *Diplodus bellottii*, *Serranus hepatus* and *Trisopterus luscus* were captured exclusively or with higher mean density and biomass on site A, corresponding to resident species that are dependent from rocky habitats (Table 4.3). On the other hand, the cartilaginous fish *Raja clavata* was only associated with sites located away from the outfall (B and C) having higher contribution for dissimilarities when measured in biomass (Table 4.3). Furthermore, the density and biomass of *Trisopteurs luscus*, *Diplodus bellotti* and *Callionymus lyra* increased with proximity to the outfall, while the species *Arnoglossus imperialis* and *Buglossidium luteum* showed the opposite pattern. Comparing the results between density and biomass data in SIMPER analyses, it is evident that large-bodied species had greater importance when the metrics are measured in biomass, which masks the influence of small-bodied species that also contribute for dissimilarities among sites (Table 4.3).

Discussion

The obtained results provided suggestive evidence that sewage discharges caused changes in both structural and functional features of fish assemblages, especially notable near the outfall. In general, the site nearest the outfall was characterized by several fish-based metrics related with species that are resident or dependent on rocky habitats. These species appear to benefit from the increased habitat complexity brought by the construction of the pipelines, which is in accordance with other studies that also detected an increase of fish abundance when the outfalls are constructed over soft-substrates (Russo 1982; Otway 1995). Actually, the pipeline constitutes a reef-like structure that provides a higher number of available resources (e.g. food and shelter) which can be exploited by species other than those characteristic of soft-substrates, providing that they are tolerant to sewage pollution (e.g. Family Serranidae). This fact explains why the metric density/biomass of rock residents was found exclusively on the outfall site.

Furthermore, some fish species may also be attracted to the plume as a possible direct or indirect source of food supply due to the increased levels of organic particulate matter or in response to increases in the abundance of their benthic prey (Russo 1982; Grigg 1994; Otway 1995; Otway et al. 1996b). Indeed, the metric density/biomass of omnivores had higher values in the site near the outfall (A), followed by site B (intermediate), and ultimately presenting the lowest values at the farthest site (C). These values are mainly due to the higher abundances of the benthopelagic species *Diplodus bellottii*, which occurs on various types of substrates. Since omnivores consist of non-specialized feeders, they are probably more able to handle changes in benthic prey availability than other trophic groups (e.g. fish or invertebrate feeders), as verified by Porter and Janz (2003) and Khalaf and Kochzius (2002). The majority of the observed fish species are macrocarnivores and invertebrate feeders. Apparently the abundances of sensitive species were balanced by some species that are more tolerant to sewage (functional redundancy), as these trophic guilds did not respond significantly to the presence of the sewage outfall despite the obtained abundance differences at the species level. In fact, besides the density/biomass of omnivores only the metric mean trophic level responded to the sewage gradient, but they were redundant.

Additionally, previous studies on sewage outfalls also have documented increases in the abundance of planktivores and detritivores (e.g. Russo 1982; Grigg 1994; Guidetti et al. 2002; Khalaf & Kochzius 2002; Guidetti et al. 2003). As the majority of the planktivores and detritivores that occur frequently in this zone are pelagic (e.g., Mugilidae*, Sardina pilchardus, Engraulis encrasicolus*) (Prista et al. 2003), it is probable that they were not caught because of the selective properties of the fishing gear. Given this, the sampling method should be complemented with other types of gears (e.g. gill nets) in order to assess the effects of sewage discharges on these trophic guilds. Despite that, the aim of this study was to characterize changes on fish assemblages associated with soft-substrates (demersal and benthopelagic species).

The direction of the response of fish assemblages to the sewage discharge (i.e. increase or decrease in abundance) is not always linear, as it depends on the rates of discharge, effluent toxicity, as well as on the characteristics of fish assemblages and heterogeneity of the environment (Russo 1982; Otway 1995; McKinley & Johnston 2010; de-la-Ossa-Carretero et al. 2012). For instance, Otway (1995) observed a general decrease in fish abundance when submarine outfalls were built over soft substrates near rocky substrates, since these already contributed greatly to habitat heterogeneity. In fact, on rocky reefs there are several examples of decreased abundance of intolerant fish species and/or

increased abundance of tolerant/opportunistic fish species (e.g. planktivores, detritivores) (Smith et al. 1999; Guidetti et al. 2002; Khalaf & Kochzius 2002; Guidetti et al. 2003; Johnston & Roberts 2009; Azzurro et al. 2010; Henriques et al. 2013). Moreover, when the effluent contains industrial wastewaters it has higher levels of contaminants, such as metals, polycyclic aromatic hydrocarbons, persistent organic pollutants and pesticides (McKinley & Johnston 2010). These contaminants are potentially toxic for fishes at certain concentrations and could affect fish assemblages by reducing fish survivorship, growth, reproductive success and prey availability, while increasing their susceptibility to diseases and deformities (see McKinley & Johnston 2010 and references therein). According to the meta-analysis of McKinley and Johnston (2010), industrial effluent reduces fish abundance (~ 50%), which contrasts with the obtained results, where total density was not significantly different among zones, probably due to the differences in the type of effluent discharged (urban wastewaters). On the other hand, the metric total biomass didn´t contribute to the discrimination among zones, being significantly different among the three zones as it is extremely dependent on fish length. In this way, none of these metrics seems to be promising to assess the effects on soft-substrate fish assemblages induced by urban wastewater discharges.

In the studied outfall, the effluent is mainly constituted by urban waste submitted to primary treatment. Therefore, an increase in organic compounds and fecal bacteria is expected (O´Sullivan 1971; Snieszko 1974). Depending not only on their concentration but also on water circulation and the initial dilution of effluent, their effects on fish assemblages could be more or less pronounced. Exophthalmus, open external sores, epitheliomas, papillomas, fin rot (necrosis), opaqueness of eyes and blindness are some of the diseases and deformities of fishes pointed out as a consequence of sewage pollution (see O´Sullivan 1971 and references therein). Previous studies performed at this outfall show that some fishes (~ 2% of total catch), especially benthic species, exhibited external deformities on the head (dorsal profile, mouth and missing one of the eyes), vertebral region and fins (absence or deformities on pectoral or caudal fins) (Santos et al. 2008). Although external deformities were not specifically addressed as an aim of this study, since it is focused on assemblage-level differences, most fishes caught near the outfall presented a generalized paleness and a characteristic odor (personal observation).

As contaminants tend to accumulate on sediments (Santos et al. 2008), it could be possible that the slightly lower densities of benthic species are related to pollution effects, explaining why the metric density/biomass of flatfishes was associated with the sites farther from the outfall, despite the fact that the same species were found in all sites

(metric with high Spearman correlation with CAP axes but without significant results in PERMANOVA). Such contaminants could also be related with the obtained results for sites placed 4 and 8 km away from the outfall, where metrics related with species that have very low or low resilience and Chondrichthyes had higher values at those sites. In general, Chondrichthyes are characterized by K-selected life histories, meaning that they have low fecundity, slow growth and late maturity (Stevens et al. 2000). These characteristics have serious implications for Chondrichthyan populations, as they limit their capacity of recovery from negative impacts. In this way, the lower densities and biomasses observed for Chondrichthyans and other species, which also have low or very low resilience (e.g. *Merluccius merluccius, Solea senegalensis*), at the outfall site may be linked to their weak recovery capacity. Furthermore, it is also possible that these species are avoiding the plumes due to a decrease in food availability or a reduction of their capacities to find prey (lower visibility and intense odor), as they are macrocarnivores that feed mostly on fish and crustaceans (Stergiou & Karpouzi 2002; Farias et al. 2006; Martinho et al. 2012). However, there are insufficient data to assess the extent to which these possible explanations contribute to the observed pattern and so further research will be needed to identify the impacts of plumes on benthic fish species (flatfish and species with low and very low resilience) and their preys.

Additionally, it is expected that young life stages (i.e. larvae, settlers and juveniles) are more sensitive to sewage than adults, as reported by Henriques et al. (2013) in rocky-reefs, unless they profit directly or indirectly from the increase in organic matter (Azzurro et al. 2010), depending on their tolerance to pollution (e.g. McKinley et al. 2011). In this way, two main hypotheses might explain the lack of response of the metric density/biomass of juveniles. Firstly, the effluent could not affect significantly the juveniles (lower toxicity and/or food improvement) or, secondly, these sites are not naturally important nursery areas.

Regarding the gradient effects of sewage, the results showed that the site positioned at an intermediate distance from the outfall (site B) showed similarities with the other two sites, being characterized by metrics that were also associated to the outfall site (density/biomass of omnivores, density/biomass of rock dependents, density/biomass of individuals with medium resilience) and with the site farthest from the outfall (density/biomass of individuals with low and very low resilience, mean trophic level and density/biomass of Chondrichthyes). In this case, species appear to benefit from some increase in resources provided by the plume, with possibly lower levels of pollution due to dilution (as explained above), which could lead to the higher values of biomass/density

observed at this site. In fact, the differences among sites were much clearer between site A (outfall) and C (8 km away), with site B (4 km away) being closer to site C.

Accounting for the fact that a higher number of metrics responded to the effect of sewage when measured with biomass data as they have into account weight differences between individuals (higher noise), that the metrics most sensitive to the gradient responded with both types of data and that the biomass data underestimated the influence of small-bodied species, it is reasonable to conclude that metrics measured in density may be better indicators of fish assemblage changes due to sewage pollution. Despite that, it is premature to conclude that density data gives always better results than biomass data, as it will be dependent on the type of pressure to be analysed and the scale needed to detect changes.

It is likely that all the above-mentioned hypotheses act synergistically and none of them alone could explain the observed patterns. In fact, the fish assemblages response to sewage results from a balance between their tolerance to pollution (toxicity), available resources, ecological characteristics and intra and inter-specific competition, sometimes without changes at the functional levels. In summary, the results showed that the metric approach provides more useful information for interpreting the consequences of human-induced changes than species individually, and reinforced the importance of using fish assemblages as biological indicators. In this context, the metrics density/biomass of individuals with low and very low resilience, density/biomass of rock residents and density/biomass of omnivores seem to be promising to detect changes on soft-substrate fish assemblages due to sewage discharge. However, it is important to note that the present study was performed in a single season/year and focusing on a single outfall discharging urban wastewaters. Consequently, despite the large size of the sampled area and the agreement with results from other studies, further effort is needed to assess the sensitivity and applicability of the selected metrics by testing the effect of variability among different seasons and years and including other types of sewage effluents (i.e. industrial). Nevertheless, the present study constituted the first functional guild approach to the effects of sewage on soft-substrate fish assemblages, contributing not only to the understanding of sewage-related impacts, but also to the future use of marine fishes as biological indicators, as required by the Marine Strategy Framework Directive.

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Supplementary data III. Database used to calculate fish-based metrics. The list presents the ecological features of each species: trophic level, length at first maturity, mobility (hm - high, mm - medium, te - territorial, se - sedentary) habitat association (R- soft-b – resident of soft-bottoms; R-rock – resident of rocky reefs; D-soft-b – dependent of soft-bottoms; D- rock – dependent of rocky reefs), trophic guilds (inv - invertebrate feeders, ma - macrocarnivores, pi - piscivores, om - omnivores, zoo - zooplanctonivores), commercial value (€ - none or low, €€ - medium, €€€ - high) and resilience (VL - very low, L - low, M - medium, H - high).

Species	Trophic level	Lmaturity	Mobility	Habitat Association				Trophic	Commercial	Resilience
				R-soft-b	R-rock	D-soft-b	D-rock			
Arnoglossus imperialis	3.8	15.7	mm	1	0	0	0	ma	€	H
Arnoglossus thori	3.3	11.3	mm	1	0	0	0	ma	€	M
Chelidonichthys cuculus	3.8	14.6	mm	0	0	1	1	ma	€	M
Buglossidium luteum	3.3	7.6	mm	1	0	0	0	inv	€€	M
Callionymus lyra	3.3	15	mm	1	0	0	1	inv	€	M
Callionymus maculatus	3.3	10.6	mm	0	0	0	0	inv	€	H
Cepola macrophthalma	3.1	42	se	1	0	0	0	zoo	€	M
Chelidonichthys lucernus	3.7	25.8	mm	1	0	0	1	ma	€€€	M
Chelidonichthys obscurus	3.4	20.7	mm	1	0	0	1	ma	€€	M
Citharus linguatula	4	15.2	mm	1	0	0	0	ma	€€	M
Dicologlossa cuneata	3.3	14.9	mm	1	0	0	0	inv	€€€	H
Diplodus bellottii	3.5	15.6	mm	0	0	0	1	om	€€€	M
Diplodus vulgaris	3.2	16.5	hm	0	0	0	1	om	€€€	H
Eutrigla gurnardus	3.6	26	mm	1	0	0	0	ma	€€	M
Lepidotrigla cavillone	3.2	7.1	mm	1	0	0	0	inv	€	H
Merluccius merluccius	4.4	60.1	mm	1	0	0	0	ma	€€€	L
Microchirus azevia	3.2	20.2	mm	1	0	0	0	inv	€€€	H
Pagellus acarne	3.5	17.7	hm	0	0	0	1	ma	€€€	M
Pomatoschistus lozanoi	3.1	5.8	se	0	0	1	1	inv	€	H
Raja clavata	3.8	45.4	mm	1	0	0	0	ma	€€€	L
Raja montagui	3.7	39.3	mm	1	0	0	0	inv	€€€	L
Rostroraja alba	4.4	120	mm	1	0	0	0	ma	€€€	L
Scomber colias	3.9	27.8	hm	0	0	0	0	ma	€€	H
Scorpaena notata	3.5	15.2	se	0	1	0	0	ma	€	M
Scyliorhinus canicula	3.7	53.5	mm	1	0	0	1	ma	€€	L
Serranus cabrilla	3.3	13.6	mm	0	1	0	0	ma	€	M
Serranus hepatus	3.5	15.7	mm	0	1	0	0	ma	€	M
Solea senegalensis	3.1	34.1	mm	1	0	0	0	inv	€€€	L
Solea solea	3.1	29.9	hm	1	0	0	0	inv	€€€	M
Spondyliosoma cantharus	3.3	31.1	hm	0	0	1	1	om	€€	M
Trachurus picturatus	3.3	34.1	hm	0	0	0	0	ma	€€	M
Trachurus trachurus	3.6	22.5	hm	0	0	0	0	ma	€€€	M
Trigloporus lastoviza	3.4	21.3	mm	1	0	0	1	inv	€€	M
Trisopterus luscus	3.7	26.4	hm	0	0	1	1	ma	€€€	M
Zeus faber	4.5	37.6	hm	0	0	0	1	ma	€€€	L

CHAPTER 5

Henriques S., Pais M.P., Vasconcelos R.P., Murta A., Azevedo M., Costa M.J. & Cabral, H.N. Structural and functional traits indicate fishing pressure on marine fish assemblages. In review in Journal of Applied Ecology.

Structural and functional traits indicate fishing pressure on marine fish assemblages

Abstract: 1. Conservation science increasingly focuses on how ecosystem functioning is affected by anthropogenic pressures, which implies an understanding about the structural and functional changes in biological assemblages and requires indicators to timely detect such changes.

2. In this sense, a novel approach was used to model the response of several trait-based metrics of fish assemblages to gradients of trawling intensity, within four distinct habitat typologies. The fishing gradient was defined based on vessel monitoring system records.

3. Overall, individuals of higher trophic levels, high commercial value, those exhibiting vulnerable traits like chondrichthyes, species with very low resilience and sedentary individuals and dominance, were the most sensitive metrics to increased level of fishing.

4. These patterns were attributed to direct and indirect fishing effects acting synergistically over specific features of fish assemblages leading to its homogenization, with likely impacts on ecosystems resilience. Since the selected metrics responded to a gradient of anthropogenic pressure, independently of the intensity levels concerned, this approach can be particular advantageous in cases where pristine conditions are absent.

5. *Synthesis and Applications.* A key goal in the proposed approach was to provide indicators that are sensitive to gradients of trawling intensity and can be extrapolated to a broader geographic region. Moreover, the identification of threshold levels of fishing pressure that fish assemblages can withstand before ecosystem functioning is altered can have deep implications on the success of management plans. In this context, a similar approach should be applied to assess other types of pressure sources and biological indicators.

Keywords: Ecosystem function, fishing gradients, fish assemblages, guild approach, marine habitats, response models, trait-based metrics.

Introduction

In recent years, structural and functional indicators of anthropogenic disturbances on marine assemblages have become an important issue in applied ecology (Bremner 2008; Auster & Link 2009; Mouillot et al. 2012). It is widely recognized that structural and functional approaches, through the analysis of trait-based metrics, have several advantages in the detection of changes in assemblage functioning, compared with strictly taxonomic-based methods, as they represent the species adaptations to the environment and their response to stress (Elliott et al. 2007; Juan et al. 2007; Noble et al. 2007; Bremner 2008; Rochet et al. 2010; Mouillot et al. 2012).

Since species have distinct sensitivities, different abundance distributions are expected under stress with some species that share traits decreasing in abundance while others remain stable or even increase (Bremner 2008; Mouillot et al. 2012). On the other hand,

some disturbances can lead to changes in species abundances without shifts within functional traits (i.e. functional redundancy), thus not affecting the assemblage's function (see Bremner 2008; and Rochet et al. 2010 for details about compensation mechanisms). Biogeographic dissimilarities in species distributions lead to regional variation in assemblages which provides little opportunity for generalization and comparison of anthropogenic effects, a problem that may be overcome by using trait-based metrics as indicators (Micheli & Halpern 2005; Elliott et al. 2007; Noble et al. 2007; Bremner 2008).

Together these facts suggest that trait-based metrics can be powerful as early warning indicators of assemblage changes in addition to making such changes easier to interpret, compare and predict in a functional perspective (Rochet & Trenkel 2003; Fulton et al. 2005; Bremner 2008; Mouillot et al. 2012), as demonstrated for benthic invertebrate assemblages (e.g. Tillin et al. 2006; Juan et al. 2007). However, while fish population-based metrics are well developed to detect fishing disturbance, considerable less attention has been given to assemblage-based metrics and, in the latter case, the use of trait-based indicators is still in its infancy (see Rochet & Trenkel 2003 for a critical review). Despite the suggestions that some structural and trophic-related metrics are good indicators of exploitation (e.g. Rochet & Trenkel 2003; Gristina et al. 2006; Methratta & Link 2006; Auster & Link 2009; Rochet et al. 2010; Dimech et al. 2012), information concerning a wide range of structural and functional traits (i.e. diversity, abundance, trophic structure, mobility, resilience) is still lacking or dispersed, which implies a poor understanding about fishing effects on assemblage functioning. Additionally, the majority of previous assessments have focused on distinct sites with different levels of fishing disturbance. Consequently, it is urgent to find indicators that are sensitive to gradients of fishing pressure in order to better understand the patterns of change and follow environmental impact gradients, which constitutes an essential property of a good indicator (Greenstreet & Rogers 2006).

By modifying seabed habitats, disrupting food web processes and removing species (e.g. target, large-bodied, vulnerable, by-catch), bottom trawling activities can have dramatic consequences on marine ecosystems (Gristina et al. 2006; Tillin et al. 2006; Juan et al. 2007; Dimech et al. 2012). This study relies on both structural and functional traits of soft-substrate fish assemblages to assess patterns of change under gradients of trawling intensity. The applied approach compared the response models of several trait-based metrics estimated from five years of scientific surveys along the Portuguese coast. The consistency of metric response among four habitat typologies allowed the selection of a set of metrics sensitive to increasing levels of fishing intensity, supporting discussion about

their usefulness as indicators of changes in assemblage function, with likely effects on marine ecosystems functioning.

Material and Methods

Study area and habitat typologies

The study area extends from 36ºN to 42ºN, covering all the Portuguese continental coast with a depth range between 20 m and 460 m (Figure 5.1). Since the distribution of marine fish species is affected by habitat features, depending on their ecological needs and physiological tolerances (Rice 2005), the study area was divided in four habitat typologies based on previous studies about the distribution patterns of fish assemblages, topographic features, oceanographic conditions (Fiúza et al. 1982; Gomes et al. 2001; Sousa et al. 2005; Figueiredo et al. 2007) and sediment charts from Hydrographic Institute of Portugal (Charts 1: 150 000): (1) North typology, continental shelf (< 200 m deep) located from Minho river to Cape Raso (38º42'0''N), which is relatively wide and flat with predominance of coarse sands and gravel substrates as well as several rocky areas; (2) Centre typology, located between Cape Raso and Cape São Vicente (37º1'30''N), also corresponds to a continental shelf section (< 200 m deep) but straighter and steeper with predominance of coarse sandy substrates and some rocky patches; (3) South typology, steeper section of the continental shelf (< 200 m deep) from Cape São Vicente to Vila Real de Santo António, characterized by fine and medium sandy sediments poorly calibrated; (4) Deep typology, continental slope throughout the coast (> 200 m deep) mainly composed of fine sands and muddy sediments.

In general, the west coast is under the influence of prevailing north and northwest winds and waves, which promote important upwelling events mainly during the summer months, while the south coast is more sheltered but highly influenced by the Mediterranean outflow (Fiúza et al. 1982; Sousa et al. 2005; Figueiredo et al. 2007). All statistical analyses were performed per habitat typology in order to minimize the confounding effects of habitat.

Trait-based metrics of fish assemblages

The fish assemblage database was compiled from a 5-year time series (2006-2010) of scientific surveys carried out by the Portuguese Institute of Sea and Atmosphere (IPMA), with the RV "Noruega", during September-October, in order to avoid the effects of seasonality. Each survey followed a mixed sampling scheme composed by 96 sampling stations (66 stations distributed over a fixed grid with 5' per 5' miles and 30 random

stations) spread throughout the study area, where 30-minute trawls were performed at a constant speed (3.5 knots) during the daylight using a bottom trawl (14 m headline; ground rope with rollers; 20 mm cod-end mesh size).

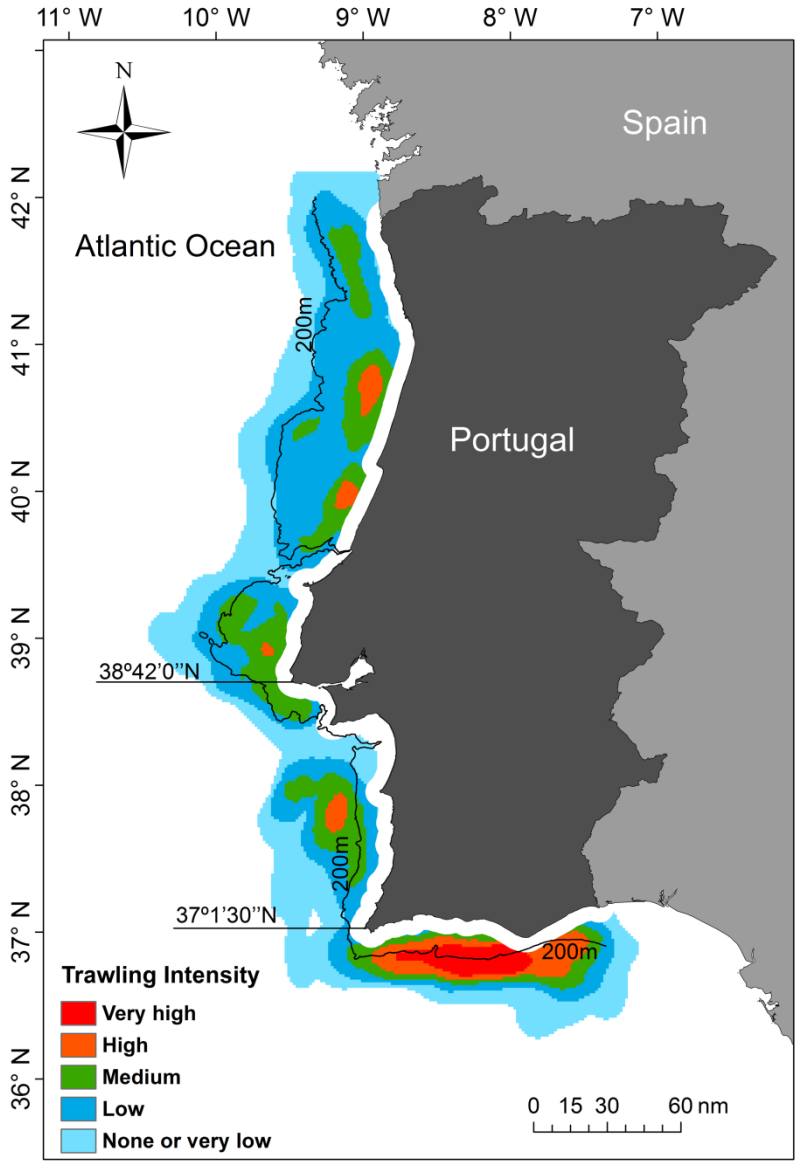

Figure 5.1 Map of the study area showing the spatial distribution of the five trawling intensity classes derived from VMS records of fishing vessels. The latitudinal borders that divide shallow habitat typologies (< 200 m deep) are marked.

In order to analyse structural and functional changes on soft-substrate fish assemblages due to trawling impacts, a set of functional traits was assigned to every species, according to the previous classification of Henriques et al. (2008) updated with available literature and FishBase online database (Froese & Pauly 2012) (Supplementary data IV), namely: trophic level, length at first maturity, mobility, trophic guild, abundance, commercial value, resilience. Afterwards, 23 trait-based metrics representing a range of fish assemblage attributes, including measures of species composition and diversity, abundance, trophic structure, resilience and mobility, were estimated per sample (haul) after standardization per unit of effort (Table 5.1). Individuals were quantified in biomass ($kg.hour^{-1}$), as it is expected to be most sensitive measure to fishing-induced changes (Harmelin-Vivien et al. 2008). Metrics were selected based on the ecological features of marine soft-substrate fish assemblages, as well as their response to anthropogenic pressures, including fishing (Rochet & Trenkel 2003; Fulton et al. 2005; Labropoulou & Papaconstantinou 2005; Greenstreet & Rogers 2006; Henriques et al. 2008; Rochet et al. 2010; Dimech et al. 2012). As pelagic species are strongly affected by climatic and environmental factors (Coll et al. 2008), are not entirely dependent from the substrate and their abundance is underestimated in bottom trawl samples (Labropoulou & Papaconstantinou 2005), they were excluded from the analyses (namely *Alosa falax*, *Auxis rochei*, *Atherina presbyter*, *Belone belone*, *Engraulis encrasicolus*, *Gadiculus argenteus*, *Liza* spp., *Macroramphosus* spp., *Mola mola*, *Micromesistius poutassou*, *Sardina pilchardus*, *Scomber* spp., *Spicara maena*, *Trachurus* spp.*, Vinciguerria poweriae* and *Xiphias gladius*).

Mapping trawling intensity

Trawling intensity was analysed using Vessel Monitoring System (VMS) data, obtained from an automated satellite-based onboard system that records time, speed and position of vessels at sea (Witt & Godley 2007; Fock 2008). Since 2005, this system is mandatory in Europe for fishing vessels larger than 15m (Witt & Godley 2007; Fock 2008), providing a robust way to spatially measure fishing intensity (e.g. Mills et al. 2007; Witt & Godley 2007; Fock 2008; Lambert et al. 2012). Using VMS data from 2006 to 2007 with records at every two hours, fishing intensity of trawlers operating in the Portuguese continental coast was estimated applying GIS techniques in ArcGIS 10.1 software. As VMS is unable to discriminate between different types of activity (e.g. steaming, fishing, in port, navigation), data were filtered by typical trawling speed (2-5 knots) in order to keep only points that likely correspond to fishing operations (Fock 2008; Alemany et al. 2012). Route lines per day and vessel were then created by joining successive position points and a

Table 5.1 List of candidate metrics to model the response of soft-substrate fish assemblages to trawling intensity and their corresponding abbreviations used in the analyses. Metrics are divided by the following attributes: diversity/composition, trophic structure, mobility, resilience.

Metric	Abbreviation	Description
Species diversity/composition/abundance		
Total number of species	Tspp	Measure of species richness
Total biomass (kg.hour^{-1})	Tbiom	Measure of abundance
Dominance	Dom	Number of species that make up 90% of the total biomass
Mean trophic level	MTL	Measures of the assemblage structure
Average fish weight	Av_weight	Measures of the assemblage structure
Biomass of individuals of rare and uncommon species	R+UC	Measures of conservation value of the system
Biomass of chondrichthyes	Chondrichthyes	Measures of conservation value of the system
Biomass of flatfish	Flatfish	Measures of conservation value of the system
Biomass of individuals with medium commercial value	€€	Measures of commercial threat
Biomass of individuals with high commercial value	€€€	Measures of commercial threat
Trophic structure		
Biomass of invertebrate feeders	inv	Feed predominantly on non-planktonic invertebrates
Biomass of omnivores	om	Feed on detritus, filamentous algae, macrophytes, epifauna and infauna
Biomass of zooplanktivores	zoo	Feed on planktonic crustacean, hydroids and fish eggs/larvae
Biomass of piscivores	pi	Feed predominantly on fish
Biomass of macrocarnivores	ma	Feed on macroinvertebrate and vertebrates (mostly fish)
Mobility		
Biomass of individuals with high mobility	hm	Movement over relatively large distances
Biomass of individuals with medium mobility	mm	Daily movement patterns on the order of tens of meters
Biomass of territorial individuals	te	Limited movement and territorial behaviour
Biomass of sedentary individuals	se	Limited movement and well defined home ranges
Resilience		
Biomass of individuals with "very low" resilience	VL	Capacity to recover from changes in the environment; Minimum population doubling time: high (up to 1.4 years), medium (1.4 to 4.4 years), low (4.5 to 14 years), very low (more than 14 years) (values available on FishBase)
Biomass of individuals with "low" resilience	L	
Biomass of individuals with "medium" resilience	M	
Biomass of individuals with "high" resilience	H	

mask of 6 nautical miles from the coast was applied to eliminate the positions where vessels were leaving or nearing ports (Witt & Godley 2007), since trawling activity is forbidden within this area according to Portuguese legislation (Portaria nº 1102-E/2000). In addition, a fishing intensity raster was created per year based on the density of route lines per grid-cell (pixel width of 1 nm^2), and the two annual rasters were then combined, cell by cell, by assigning their mean value into a final raster output (ArcGIS multiple raster operation tools). Finally, 5 classes of fishing intensity were defined (i.e. 1 - none or very low, 2 - low, 3 - moderate, 4 - high and 5 - very high) using Jenks natural break classification. This classification method outlines the best arrangement among classes by seeking to reduce the variance within classes while maximizing the variance between classes (Alemany et al. 2012). Each sample was then classified according to this scale.

Statistical analyses

Mapping of VMS data highlighted considerable heterogeneity in the fishing intensity among the different typologies, with the south coast being the most intensively trawled (Figure 5.1). Therefore, the data were analysed according to a gradient of fishing pressure and not according to the categorical division of the different fishing classes.

Before identifying sensitive metrics, several preliminary analyses were done in order to accomplish the assumptions of the linear modelling analyses (Anderson et al. 2008). For each habitat typology, Draftsman plots were drawn to visually assess when the metric distribution was notably skewed and to detect cases of multi-collinearity (Clarke & Gorley 2006; Anderson et al. 2008). Right-skewed metrics were square-root transformed and only one of the metrics from redundant pairs ($|r| \geq 0.95$) was retained as a proxy for the other (Clarke & Gorley 2006; Anderson et al. 2008). Moreover, two metrics (biomass of piscivores and biomass of territorial individuals) were excluded from the analyses as they were only present in very few samples and are not characteristic of these assemblages. Extreme multivariate outliers were also identified and removed through the observation of Principal Coordinates Analysis (PCO) plots based on Euclidean distances among all pairs of samples with all metrics previously normalized to place them on a comparable measurement scale (Anderson et al. 2008). The resultant datasets for the groups of replicate samples (North, Centre, South and Deep habitat typologies) comprised non-redundant and non-skewed metrics, samples without extreme outliers and representative metrics.

For each habitat typology, non-parametric distance-based linear models (DISTLM; Anderson et al. 2008) were used to identify the relationship between trait-based metrics (used as predictor variables) and the gradients of trawling intensity (coded as model matrices). A metric-selection approach inspired by the method proposed by Hallett et al. (2012) was applied: (1) Model matrices were built, so that the distances among pairs of samples reflected the order of intensity of the defined classes (e.g. same-class samples with distance 0, category 5 samples with distance 3 from category 2 samples) (Clarke & Gorley 2006); (2) Distance-based linear models were then run to select the best subset of trait-based metrics by testing all possible combinations and computing the corrected Akaike Information Criterion (AIC_c), developed for cases where the number of samples (n) relative to predictor variables (q) is small (Anderson et al. 2008 and references therein); (3) The selection of the best model for each typology was done by seeking the compromise between the lowest AIC_c value, higher proportion of the explained variation (R^2) and lower number of metrics. The final set of metrics that best responded to changes in trawling intensity categories were selected if they were part of the best model in at least half of the typologies and if they had a predictable steering response (i.e. increase or decrease).

In order to complement the information provided by the models and given that the model approach is not suitable to apply with species data, due to high number of species compared with samples, a SIMPER routine using Bray-Curtis dissimilarities was used to identify species that contributed most for the biomass dissimilarities between trawling intensity classes within each typology (North, Centre, South and Deep). Bray-Curtis dissimilarities were computed after square root transformation of biomass data to reduce the influence of dominant species.

All statistical analyses were performed using PRIMER 6 package with PERMANOVA+ (Clarke & Gorley 2006; Anderson et al. 2008).

Results

The DISTLM results showed that several trait-based metrics responded to increasing levels of fishing intensity (Table 5.2). The goodness of fit of the models was generally high, with the highest percentages of explained variation corresponding to the Centre and South typologies (66.1% and 67.4%, respectively) followed by the Deep typology (40.7%) and with the lowest value in the North typology (27.4%). Although the number of metrics selected in each model varied among typologies, some metrics were consistently selected (i.e. at least by half of the models) (Table 5.2). These include dominance, mean trophic

level, chondrichthyes, invertebrate feeders, macrocarnivores and individuals with very low, low and medium resilience. However among these, only those that showed a predictable trend (i.e. increase or decrease) were selected as suitable to detect differences between categorical levels of trawling intensity (Table 5.2). Therefore, the metrics low and medium resilience and the metric mean trophic level were excluded. The majority of metric values decreased with an increase in trawling intensity, which in the case of the dominance means that the assemblage is progressively dominated by few species with the increasing fishing intensity.

Table 5.2 Results of the DISTLM models for each habitat typology (North, Centre, South and Deep). Shown is the response trend of the trait-based metrics ("+" increase; "-" decrease) along the gradient of trawling intensity (1 - none or very low; 2 - low; 3 - medium; 4 - high; 5 - very high) in the best model. Metric trends per typology were based on mean values observed in each trawling intensity class. Redundant metrics in bold were the ones used as proxies in the analyses. Goodness of fit of models is also shown: percentage of explained variation and AIC_c. Only the metrics selected by the models are shown. See Table 5.1 for metric abbreviations.

Metrics	**North** (2;3;4)	**Centre** (1;2;3)	**South** (1;3;4;5)	**Deep** (1;2;3;4;5)
Tspp	-			
Dom		+	+	
MTL			+	-
R+UC				-
Chondrichthyes		-	-	-
€€€			-	
inv		-	-	-
om			-	
zoo	+			
ma	-	-		
se				-
VL	-			-
L	-	+	+	
M	-			+
Redundancy among metrics	Tbiom/**ma**/€€€; mm/**L**; **VL**/se; Av_weight/Dom/**MTL**	Tbiom/**inv**/mm/H; **ma**/€€€; hm/**M**	Tbiom/hm/**€€€**; mm/**L**	Tbiom/**inv**/mm/H; **ma**/€€€; hm/**M**
% of explained variation by the best solution model	**27.1%**	**66.1%**	**67.4%**	**40.7%**
Selection criterion (AICc)	**-188.3**	**-72.15**	**-15.26**	**4.82**

Besides these metrics, it is also important to analyse redundant metrics that were excluded from the model analyses in order to verify if their response is comparable to their proxies (Table 5.2). The metric biomass of individuals with high commercial value was frequently redundant with biomass of macrocarnivores and the biomass of sedentary individuals with the biomass of individuals with very low resilience. Moreover, the metric total biomass was always redundant with one of the metrics chosen by the models.

Some metrics were only chosen by one of the models and were not selected for the final set, as they probably represent specific features of a particular typology (total number of species, rare and uncommon individuals, omnivores and zooplanktivores), whereas other metrics (medium commercial value, flatfish, average fish weight, high mobility and high resilience) were never included in the best models and were rarely selected when correlated to other metrics (e.g. medium mobility) (Table 5.2).

In summary, out of 21 trait-based metrics tested, only 8 showed consistent responses to differences in trawling intensity. These include the dominance, biomass of chondrichthyes, biomass of invertebrate feeders, biomass of macrocarnivores, biomass of sedentary individuals, biomass of individuals with very low resilience, biomass of individuals with high commercial value and total biomass, all of which decreased as fishing intensity increased (Table 5.2; Figure 5.2).

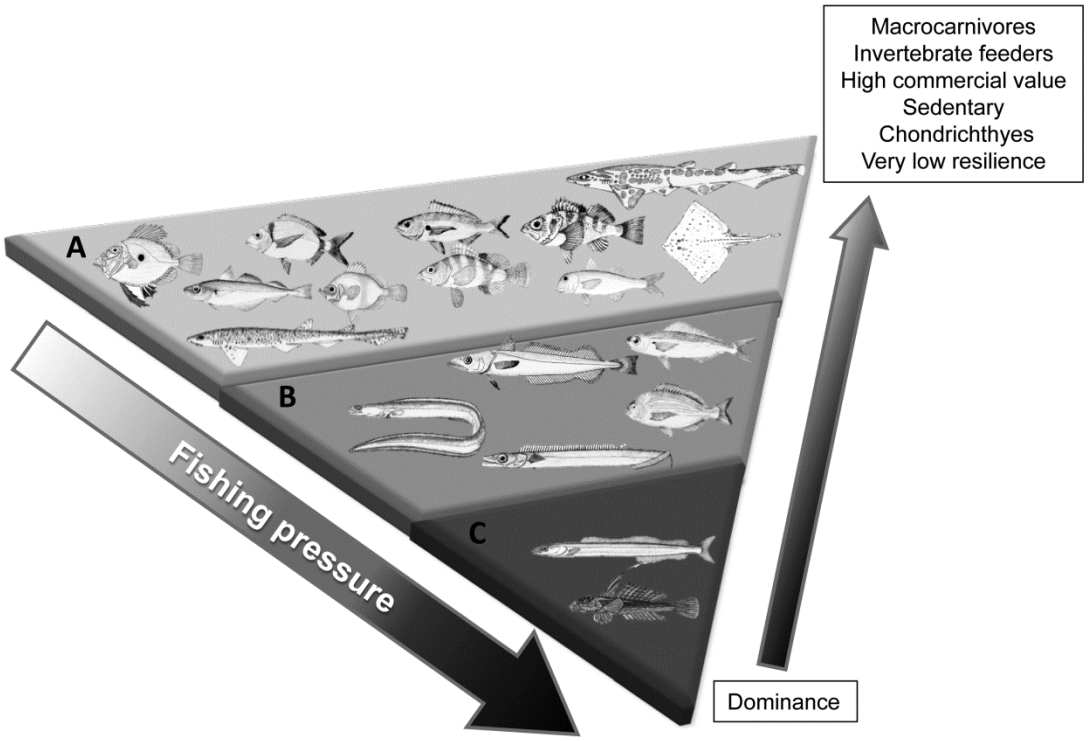

Figure 5.2 Diagram illustrating the response trend of the selected trait-based metrics and fish species according to increasing levels of fishing intensity. Biomass of species from group A generally decreased with increasing intensity, the response of species from group B was not consistent and the biomass of species from group C increased. For species names see Table 5.3.

Table 5.3 Results of the SIMPER analysis for each habitat typology (North, Centre, South and Deep). Shown is the response trend of the species that most contributed (90%) for the differences between trawling intensity classes (1 - none or very low; 2 - low; 3 - medium; 4 - high; 5 - very high). Group A - species which decreased in biomass (-) with increasing levels of trawling intensity; Group B - species that did not show a consistent trend (±); Group C - species which increased in biomass with increasing levels of trawling intensity.

Group	Species	North (2;3;4)	Centre (1;2;3)	South (1;3;4;5)	Deep (1;2;3;4;5)
A	*Capros aper*	-	-	-	-
	Diplodus vulgaris			-	
	Galeus melastomus				-
	Helicolenus dactylopterus	-			-
	Lepidotrigla cavillone			-	
	Lophius piscatorius				-
	Mullus spp.		-	-	
	Pagellus acarne	-	-	-	
	Pagrus pagrus		-		
	Phycis blennoides				-
	Raja spp.	-	-	-	
	Scorpaena notata			-	
	Scyliorhinus canicula	-	-	-	-
	Serranus hepatus			-	
	Trisopterus luscus	-	-		
	Zeus faber	-	-	-	
B	*Boops boops*	-	-	±	
	Conger conger	-	+	-	±
	Lepidopus caudatus		+	-	+
	Merluccius merluccius	-	+	±	-
	Spondyliosoma cantharus	+	±	±	
C	*Callionymus lyra*	+			
	Hyperoplus lanceolatus	+			

SIMPER evidenced the general response trends of fish species biomass per habitat typology (Table 5.3), i.e. the species that most contributed to differences between trawling intensity classes were identified. Three main groups of species are observed according to their response to different categories of fishing intensity. Group A comprised species whose biomass generally decreases with increasing fishing intensity, group B included species that not always have a predictable response and group C included two species that respond positively to increasing pressure in the North typology. Most of these species have high commercial value and the majority belongs to group A, including all the chondrichthyes. Group A also comprises many of the sedentary species, invertebrate feeders and a wide range of species with different classes of resilience, while species from group B have higher mobility and most have medium resilience. Besides, all species from group B have gregarious behaviour, with exception of *Conger conger*, which depends on

the proximity of rocky patches. Regardless of these results, it is important to highlight that the metrics were estimated based on the total 119 fish species included in this study, and although these species were those that most contributed for differences between trawling intensity classes, they are not fully representative of the fishing-induced changes as measured with trait-based metrics.

Discussion

Several trait-based metrics were identified as sensitive to differences in trawling intensity, indicating that trawling caused changes on both structural and functional aspects of soft-substrate fish assemblages. The consistent changes on some of the trait-based metrics and fish species in most of the habitat typologies analysed suggests that trawling pressure was the main factor responsible for these changes, rather than other environmental constraints, such as habitat and inter-annual variability (along the 5 years studied). These results are in accordance with models of Coll et al. (2008), which identified fishing as the main impact driving the dynamics of demersal species, while environmental driving forces were predominant in the pelagic system.

A general response trend to fishing gradients was found for all typologies, even if only small differences exist between adjacent fishing classes. Nevertheless, it should be noted that not all trawling intensity classes were present in every typology, which could in part explain metrics showing an inconsistent response or selected in just one model, as well as species with variable trends in biomass. Additionally, in the North typology, the best model only explained 27% of total variation, which could be associated with heterogeneity due to the environmental particularities of this typology, as it is more exposed to severe oceanographic conditions and has a wider and flatter shelf (Fiúza et al. 1982; Gomes et al. 2001; Figueiredo et al. 2007). Still, several trait-based metrics followed the same trend of the remaining typologies.

Trawling fisheries are characterized by their low selectivity with low by-catch survival and a great physical damage to habitat, which often results in heavily exploited areas being dominated by few opportunistic/tolerant species (Tillin et al. 2006; Juan et al. 2007; Kaiser & Hiddink 2007; Dimech et al. 2012). Such fishing method can have both direct and indirect effects that ultimately change the ecosystem structure and function through dynamic processes of bottom-up, wasp-waist and top-down control (Caddy & Garibaldi 2000; Cury et al. 2001). Present results support these predictions as they indicate trawling-driven declines in total biomass, with particular incidence in the biomass of

macrocarnivores, invertebrate feeders, chondrichthyes, sedentary species, species with high commercial value and individuals with very low resilience, along with an increase in dominance.

Changes in total biomass can be difficult to predict because of the indirect effects along food webs (e.g. depletion of top-predators and eutrophication) and environmental variability (e.g. in upwelling systems), leading to fluctuations in the abundance of small-sized pelagic and demersal fish species with gregarious behaviour (e.g. Farina et al. 1997; Rogers & Ellis 2000; Coll et al. 2008; Barausse et al. 2011). Therefore, here the predictable trend in total biomass was probably detectable due to the exclusion of pelagic species. Moreover, total biomass was redundant with many other metrics and the meaning of their increase or decrease is difficult to understand when used alone. These facts suggest that the metric total biomass should be used with caution and the analysis of assemblage components may lead to clearer information (see Caddy & Garibaldi 2000; and Rochet & Trenkel 2003).

The majority of analysed species were macrocarnivores (including most species with high commercial value) or invertebrate feeders. Their decrease is directly related with depletion from commercial fishing and probably indirectly related to physical disturbance on the benthic ecosystem. Indeed, increased proportions of burrowers and opportunistic invertebrate scavengers have been pointed out as functional changes following trawling disturbance, with consequent loss of functional diversity and dominance by smaller and short-lived species in benthic invertebrate assemblages (e.g. Tillin et al. 2006; Juan et al. 2007; Kaiser & Hiddink 2007; Dimech et al. 2012). However, while some sensitive invertebrate species are simply removed, in other cases no changes in the abundance of opportunistic species were reported (Kaiser & Hiddink 2007) despite changes in their proportion, and therefore, although the dominance of a particular benthic prey could be beneficial to its predators, an increase in abundance of great magnitude is not expected. Moreover, the higher diversity of benthic invertebrates in less disturbed areas may better fulfil the resource requirements of a broader range of fish species (Juan et al. 2007), explaining, together with fishing mortality, the decreasing trend in biomass of macrocarnivores and invertebrate feeders resulting from trawling-induced habitat homogeneity. Such high vulnerability to direct and indirect impacts suggests that these trophic groups may lose some species in chronically trawled areas, with consequent loss of functional diversity and ultimately leading to degradation of ecosystem functions (Micheli & Halpern 2005; Juan et al. 2007). There is, however, a considerable lack of information about the role of macrocarnivores and invertebrate feeders in maintaining ecosystem

functions on soft-substrate habitats, but it is likely that their effects are centred on the abundance and composition of invertebrate assemblages, which in turn are crucial elements of key biochemical processes such as nutrient cycling (Allen & Clarke 2007).

Conversely, omnivores are probably more able to handle changes in benthic prey availability than other trophic groups, as they are non-specialized feeders by definition (Khalaf & Kochzius 2002). In this case, the direct effect of fishing mortality on some omnivore species is balanced by other species with high resilience to fishing (functional redundancy), therefore no consistent response was found for this trophic guild. In fact, only three species of omnivores contributed most for the differences between trawling intensity classes and the response of the gregarious species *Boops boops* and *Spondyliosoma cantharus* (with medium commercial value) was inconsistent.

Although the mean trophic level has been pointed out as a promising indicator of fishing impacts (Rochet & Trenkel 2003), it showed an inconsistent trend, which could be attributed to the characteristics of the analysed fish assemblages. Actually, the south coast is characterized by high densities of omnivore species (i.e. from the Sparidae family) (Gomes et al. 2001; Sousa et al. 2005), and some of them constitute important target species for fish trawlers (Costa et al. 2008). This justifies why only in this typology the biomass of omnivores was selected in the model and why the mean trophic level increased with increasing trawling intensity, as omnivores have lower trophic levels than macrocarnivores. Consequently, it is possible that long-term changes (over decades) in fish assemblages lead to a general decrease in mean trophic level (e.g. Jennings et al. 2002; Coll et al. 2008), but this was not detected at the temporal and spatial scale addressed in the present study.

As broadly demonstrated worldwide, chondrichthyes showed a very consistent decreasing trend with increasing trawling intensity. Due to their specific life-history strategy, characterized by slow growth, late maturity, long life spans and low fecundity (K-strategy), chondrichthyes are particularly vulnerable to fishing (Stevens et al. 2000; Gristina et al. 2006). In the studied area, cartilaginous fishes are captured by both crustacean and fish trawlers, representing an important proportion of commercial by-catch (Costa et al. 2008), thus decreases in mean weight and distribution patterns of most commercially important rays have been already reported (Figueiredo et al. 2007). Furthermore, the effects of fishing are dependent on the balance between fishing intensity and species vulnerability to disturbance (e.g. Gristina et al. 2006). Hence, the decreasing trend observed in the biomass of species with very low resilience and of sedentary individuals (metrics that were

redundant in one typology) can be linked to their weak capacity to recover, associated to their limited movement beyond home ranges, as in *Helicolenus dactylopterus* (e.g. Dimech et al. 2012). In addition, changes in some species with low resilience, such as the majority of chondrichthyes, were balanced by other species that, despite their low resilience, probably have other features that make them less sensitive to fishing (e.g. high and medium mobility) resulting in an inconsistent trend response of the metric. These results highlight the advantages of using trait-based indicators in the assessment of anthropogenic disturbances (Bremner 2008), as they permit the identification of assemblage features that are more sensitive to changes.

Results from trait-based metrics were also supported by underlying species responses, as species which decreased in biomass with increasing levels of trawling intensity (group A response type), showed an identical trend when subject to similar pressure in other areas (e.g. Gristina et al. 2006; Dimech et al. 2012). In this context, the biomass of macrocarnivores (or individuals with high commercial value), invertebrate feeders, chondrichthyes, individuals with very low resilience (or sedentary) and dominance seem to be useful metrics to follow changes in soft-substrate fish assemblages. In summary, both trait-based metrics and species biomass data pointed to a pattern of ecosystem degradation due to trawling activities, with synergistic processes acting over structural and functional features of soft-substrate fish assemblages, leading to their homogenization and consequent dominance by fewer species. As the stability of structural and functional groups depends on the diversity of life history strategies, through density-dependent compensation by resilient members (functional redundancy) (Tillin et al. 2006; Bremner 2008; Rochet et al. 2010), the homogeneity of fish assemblages can have a profound impact on the ecosystem resilience, making it more vulnerable (Elmqvist et al. 2003). Although the approach presented does not directly measure ecosystem functioning, which is determined by a complex interaction of physical, chemical and biological components (Bremner 2008), it offers clearer insights into how ecosystems are changing, since it focuses on the structural and functional trends of assemblages instead of species alone (Mouillot et al. 2012).

A key goal in the proposed approach was to provide indicators that are sensitive to gradients of trawling intensity and can be extrapolated to a broader geographic region. As long-term changes over decades appear to be strongly influenced by fluctuations on oceanographic conditions, such as climatic oscillations and changes (Farina et al. 1997), these type of indicators (sensitive to gradients) can also be useful to improve our knowledge about fishing-induced changes, constituting a useful complement to traditional

stock assessments and long-term studies. Since the selected metrics responded to a gradient of anthropogenic pressure, independently of the intensity levels concerned, this approach can be particular advantageous in cases where pristine conditions are absent. Finally, the identification of threshold levels of fishing pressure that fish assemblages can withstand before ecosystem functioning is altered can have deep implications on the success of management plans. In this context, a similar approach should be applied to assess other types of pressure sources.

Acknowledgements

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Supplementary data IV. Database used to calculate fish-based metrics. The list presents the ecological features of each species: trophic level, mobility (hm- high, mm- medium, te- territorial, se- sedentary), trophic guilds (inv- invertebrate feeders, ma- macrocarnivores, pi- piscivores, om- omnivores, zoo- zooplanktivores), commercial value (€- none or low, L-low, M- medium, H- high) and resilience (H - high, M - medium, L - low, VL – very low).

Species	Family	Trophic level	Mobility	Trophic	Abundance	Commercial	Resilience
Ammodytes tobianus	Ammodytidae	3,2	te	zoo	VC	€	H
Gymnammodytes semisquamatus	Ammodytidae	2,7	mm	zoo	UC	€	M
Hyperoplus lanceolatus	Ammodytidae	4,2	hm	ma	C	€	M
Argentina sphyraena	Argentinidae	3,6	mm	ma	C	€	M
Beryx decadactylus	Berycidae	4,3	mm	ma	UC	€€	L
Beryx splendens	Berycidae	4,4	mm	ma	UC	€€€	L
Blennius ocellaris	Blenniidae	3,5	te	om	UC	€	M
Arnoglossus imperialis	Bothidae	3,8	mm	ma	C	€	H
Arnoglossus laterna	Bothidae	3,6	mm	ma	C	€	M
Arnoglossus rueppelii	Bothidae	4	mm	ma	R	€	M
Arnoglossus thori	Bothidae	3,3	mm	ma	UC	€	M
Brama brama	Bramidae	4,1	hm	ma	UC	€€€	L
Callanthias ruber	Callanthiidae	3,8	mm	ma	R	€	M
Callionymus lyra	Callionymidae	3,3	mm	inv	C	€	M
Callionymus maculatus	Callionymidae	3,3	mm	inv	UC	€	H
Synchiropus phaeton	Callionymidae	3,4	mm	inv	UC	€	H
Capros aper	Caproidae	3,1	mm	inv	C	€	H
Centrolophus niger	Centrolophidae	3,9	hm	ma	R	€	
Cepola macrophthalma	Cepolidae	3,1	se	zoo	C	€	M
Chaunax pictus	Chaunaciidae	4,3	mm		UC	€	M
Chimaera monstrosa	Chimaeridae	3,5	hm	inv	R	€	L
Chlorophthalmus agassizi	Chlorophthalmidae	3,7	mm	zoo	?	€	M
Citharus linguatula	Citharidae	4	mm	ma	C	€€	M
Conger conger	Congridae	4,3	hm	ma	C	€€€	VL
Symphurus nigrescens	Cynoglossidae	3,3	mm	inv	R	€€	H
Epigonus denticulatus	Epigonidae	3,3		zoo		€	M
Etmopterus pusillus	Etmopteridae	4,2	hm	ma	R	€€	L
Etmopterus spinax	Etmopteridae	3,8	hm	ma	UC	€€	VL
Trisopterus luscus	Gadidae	3,7	hm	ma	VC	€€€	M
Trisopterus minutus	Gadidae	3,8	mm	ma	C	€€€	M
Gobiidae	Gobiidae	3	te	om	VC	€	M
Lesueurigobius friesii	Gobiidae	3,2	te	inv	R	€	M
Lesueurigobius sanzi	Gobiidae	3,6	te	inv	R	€	H
Pomatoschistus minutus	Gobiidae	3,2	hm	inv	C	€	H
Acantholabrus palloni	Labridae	3,5	mm	inv	UC	€	M
Ctenolabrus rupestris	Labridae	3,3	mm	inv	VC	€	M
Labrus bergylta	Labridae	3,1	mm	inv	VC	€€	L
Labrus mixtus	Labridae	3,9	mm	inv	C	€€	L
Lophius budegassa	Lophiidae	4,5	se	ma	UC	€€€	M
Lophius piscatorius	Lophiidae	4,4	se	ma	C	€€€	L
Gaidropsarus biscayensis	Lotidae	3,6	mm	ma	UC	€	M
Gaidropsarus mediterraneus	Lotidae	3,4	hm	om	UC	€	L
Gaidropsarus vulgaris	Lotidae	3,3	mm	ma	UC	€	L
Caelorinchus caelorhincus	Macrouridae	3,6	mm	ma	R	€	L
Hymenocephalus italicus	Macrouridae	3,4		zoo		€	M
Malacocephalus laevis	Macrouridae	4,2	mm	inv	R	€	L
Nezumia bairdii	Macrouridae		mm	inv		€	M

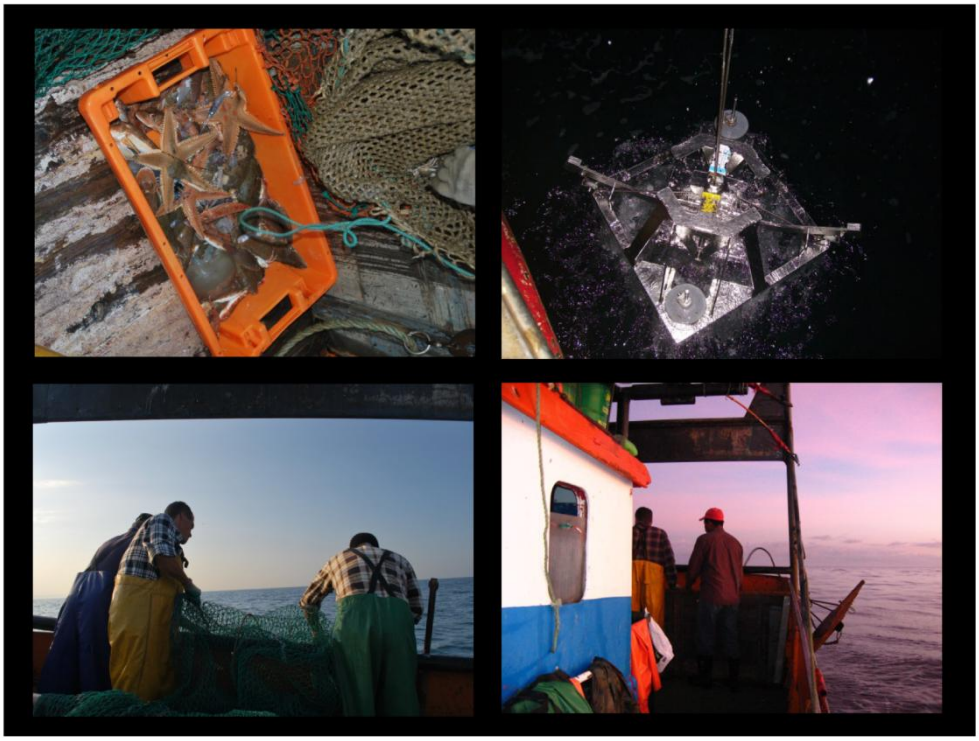
Supplementary data IV. (continued)

Species	Family	Trophic level	Mobility	Trophic	Abundance	Commercial	Resilienc
Merluccius merluccius	Merlucciidae	4,4	mm	ma	C	€€€	L
Mullus barbatus	Mullidae	3,2	mm	inv	VC	€€€	M
Mullus surmuletus	Mullidae	3,4	hm	inv	VC	€€€	M
Nettastoma melanurum	Nettastomatidae	3,5	se	ma	R	€	L
Cyttopsis rosea	Parazenidae	4	mm	ma	R	€	
Peristedion cataphractum	Peristediidae	3,5	mm	inv		€	M
Polymetme corythaeola	Phosichthydae	4,4	mm	pi		€	H
Phycis blennoides	Phycidae	3,7	mm	inv	C	€€€	M
Phycis phycis	Phycidae	4,3	mm	inv	C	€€€	M
Platichthys flesus	Pleuronectidae	3,2	hm	ma	C	€€€	M
Polyprion americanus	Polyprionidae	3,8	mm	ma	UC	€€€	L
Leucoraja naevus	Rajidae	3,9	mm	ma	UC	€€€	L
Raja brachyura	Rajidae	4	mm	ma	C	€€€	L
Raja clavata	Rajidae	3,8	mm	ma	VC	€€€	L
Raja microocellata	Rajidae	3,9	mm	pi	C	€€€	L
Raja miraletus	Rajidae	3,8	mm	ma	C	€€€	L
Raja montagui	Rajidae	3,7	mm	inv	C	€€€	L
Raja undulata	Rajidae	3,5	mm	ma	VC	€€€	L
Lepidorhombus boscii	Scophthalmidae	3,7	mm	ma	UC	€€€	M
Lepidorhombus whiffiagonis	Scophthalmidae	4,2	mm	ma	C	€€€	L
Scophthalmus maximus	Scophthalmidae	4	hm	ma	C	€€€	M
Scophthalmus rhombus	Scophthalmidae	3,8	hm	ma	VC	€€€	M
Scorpaena notata	Scorpaenidae	3,5	se	ma	VC	€	M
Scorpaena scrofa	Scorpaenidae	4,3	se	ma	VC	€	H
Galeus melastomus	Scyliorhinidae	4,2	mm	ma	UC	€€	L
Scyliorhinus canicula	Scyliorhinidae	3,7	mm	ma	C	€€	L
Helicolenus dactylopterus	Sebestidae	3,8	se	ma	C	€€€	VL
Anthias anthias	Serranidae	3,8	mm	zoo	UC	€	M
Serranus cabrilla	Serranidae	3,3	mm	ma	VC	€	M
Serranus hepatus	Serranidae	3,5	mm	ma	C	€	M
Bathysolea profundicola	Soleidae	3,2	mm	inv	R	€€€	H
Dicologlossa cuneata	Soleidae	3,3	mm	inv	VC	€€€	H
Microchirus azevia	Soleidae	3,2	mm	inv	C	€€€	H
Microchirus boscanion	Soleidae	3,2	mm	inv	R	€	H
Microchirus variegatus	Soleidae	3,3	mm	inv	C	€€€	M
Monochirus hispidus	Soleidae	3,5	mm	inv	C	€€	H
Solea lascaris	Soleidae	3,2	mm	inv	VC	€€€	M
Solea senegalensis	Soleidae	3,1	mm	inv	VC	€€€	L
Solea solea	Soleidae	3,1	hm	inv	VC	€€€	M
Scymnodon ringens	Somniosidae	3,9	mm	ma	R	€	L
Boops boops	Sparidae	3	hm	om	C	€€	M
Diplodus annularis	Sparidae	3,4	mm	om	C	€€€	M
Diplodus bellottii	Sparidae	3,5	mm	om	VC	€€€	M
Diplodus vulgaris	Sparidae	3,2	hm	om	VC	€€€	H
Pagellus acarne	Sparidae	3,5	hm	ma	VC	€€€	M
Pagellus bogaraveo	Sparidae	3,5	mm	ma	C	€€€	L
Pagellus erythrinus	Sparidae	3,4	hm	ma	C	€€€	M
Pagrus auriga	Sparidae	3,4	hm	inv	UC	€€€	VL
Pagrus pagrus	Sparidae	3,7	hm	ma	C	€€€	M
Sarpa salpa	Sparidae	2	mm	he	VC	€€	M
Spondyliosoma cantharus	Sparidae	3,3	hm	om	VC	€€	M

Supplementary data IV. (continued)

Species	Family	Trophic level	Mobility	Trophic	Abundance	Commercial	Resilience
Argyropelecus hemigymnus	Sternoptychidae	3,3	hm	zoo	R	€	H
Maurolicus muelleri	Sternoptychidae	3	mm	inv	UC	€	M
Hippocampus hippocampus	Syngnathidae	3,2	se	zoo	UC	€	H
Nerophis ophidion	Syngnathidae	4	se	inv	C	€	H
Sphoeroides pachygaster	Tetraodontidae	4,2	mm	inv	R	€	M
Torpedo marmorata	Torpedinidae	4,5	mm	ma	C	€€	L
Hoplostethus atlanticus	Trachichthyidae	4,3	se	ma	R	€	VL
Hoplostethus mediterraneus	Trachichthyidae	3,5	se	ma	R	€	L
Echiichthys vipera	Trachinidae	4,4	se	ma	VC	€	H
Trachinus draco	Trachinidae	4,2	se	ma	C	€	M
Lepidopus caudatus	Trichiuridae	3,8	hm	ma	C	€€€	M
Chelidonichthys cuculus	Triglidae	3,8	mm	ma	C	€	M
Chelidonichthys lucernus	Triglidae	3,7	mm	ma	C	€€€	M
Chelidonichthys obscurus	Triglidae	3,4	mm	ma	C	€€	M
Eutrigla gurnardus	Triglidae	3,6	mm	ma	C	€€	M
Lepidotrigla cavillone	Triglidae	3,2	mm	inv	C	€	H
Lepidotrigla dieuzeidei	Triglidae	3,3	mm	inv	UC	€	H
Trigla lyra	Triglidae	3,5	mm	inv	C	€€€	M
Trigloporus lastoviza	Triglidae	3,4	mm	inv	C	€€	M
Zenopsis conchifer	Zeidae	4,5	mm	pi	UC	€	L
Zeus faber	Zeidae	4,5	hm	ma	C	€€€	L

CHAPTER 6



Henriques S., Pais M.P., Batista M.I., Teixeira C.M., Costa M.J. & Cabral H.N. Can different biological indicators detect similar trends of marine ecosystem degradation? Submitted to Ecological Indicators.

Can different biological indicators detect similar trends of marine ecosystem degradation?

Abstract: Marine ecosystems are typically under the influence of multiple human pressure sources, which hinders the assessment of pressure-specific effects upon their biological assemblages. In this context, distance-based linear models were used to analyse the relationships of several trait-based metrics of macroinvertebrates and fish with the pressure-specific types (i.e. fishing, organic, physical and non-point-source) and global pattern of cumulative pressures. Both indicators detected similarly the effects of the global degradation and the analyses of the metrics' sensitivity (given the expected response trends) suggested that the non-point-source had the strongest contribution to this pattern, followed by organic pollution. The difficulties of assessing single pressure effects in a multiple pressure context are discussed. An approach based on the previous identification of pressure sources, a sampling strategy directed to those sources, together with indicator response is highly recommended, as it could be the only way to accurately predict human-induced changes on broad range ecosystems, with likely implications in the success of marine management plans.

Keywords: Benthic macroinvertebrate assemblages, fish assemblages, marine soft-substrates, trait-based metrics, structural and functional approach, Human Pressure Index, anthropogenic pressures.

Introduction

Awareness of the harmful effects of human pressures on the marine environment has resulted in an increasing attention to monitoring using biological indicators, in order to identify which human pressures are driving changes on the ecosystem structure and function, as well as design management plans to minimize impacts (Niemi et al. 2004; Rogers & Greenaway 2005; Smale et al. 2010). In this context, recent politics have been developed with the purpose of promoting sustainable use of marine resources and protect marine ecosystems (e.g. Marine Strategy Framework Directive, MSFD; Directive 2008/56/CE). To implement the MSFD, an integrated ecosystem-based approach should be applied, giving priority to the attainment of a "good environmental status" through the assessment of physical and chemical elements, together with several biological indicators, among which are fish and macroinvertebrates (see annex III in Directive 2008/56/CE).

Due to the difficulty of analysing patterns of change in complex, spatially and temporally diverse multi-species assemblages, the need to assess environmental status comes with new challenges concerning the use of biological indicators in marine waters (Niemi et al. 2004; Niemi & McDonald 2004; Mee et al. 2008). Additionally, stress in marine ecosystems is usually characterized by the effects of multiple human pressure sources, and as physical boundaries between marine habitats are difficult to define, thus the identification of

pressures that are affecting an area constitutes a complex task (Niemi et al. 2004; Ban et al. 2010). This way, coupling human pressure and biological response analyses is essential to link the causes of stress to the response of indicators. Otherwise, it would be extremely difficult to identify sources of disturbance, unless pressure-specific metrics exist and detect such changes (Niemi et al. 2004; Niemi & McDonald 2004).

Earlier attempts at comparing the response of fish-based and macroinvertebrate-based metrics have been focused on freshwater ecosystems (e.g. Hering et al. 2006; Johnson et al. 2006; Marzin et al. 2012). In general, these studies showed that macroinvertebrates and fish have different sensitivities depending on the human pressure analysed, with fish responding more to hydrological changes, while macroinvertebrates show a higher sensitivity to water quality and/or geomorphological changes (Hering et al. 2006; Marzin et al. 2012). However, these assemblages differ deeply from those of marine waters. For example, fish assemblages are known to be species-poor in streams (Hering et al. 2006). To our knowledge, only few studies have compared the response of multiple indicators in coastal waters (marine and estuarine ecosystems), but through multimetric indices (e.g. Borja et al. 2009; Azevedo et al. 2011). Therefore, a complete approach based on structural and functional metrics is still lacking. Despite that, these studies showed that both fish and macroinvertebrates indices had a consistent response to water quality improvement (Borja et al. 2009) and in the detection of degraded sites (Azevedo et al. 2011).

Although it seems that both biological indicators (i.e. fish and macroinvertebrates) are capable of detecting ecosystem degradation, they have completely different biological traits. Fish have longer life cycles, occupy a variety of trophic levels (reflecting effects at all levels within food webs) and higher mobility (although some species have limited ranges), which probably makes them more sensitive to large-scale changes (Whitfield & Elliott 2002; Elliott et al. 2007). Compared with fish, benthic macroinvertebrates have short life cycles and are relatively sedentary, which makes them more vulnerable to small variations in the ecosystem (Aarnio et al. 2011; Marzin et al. 2012). Based on these assumptions, it would be expected that these biological assemblages have different sensitivities to disturbance.

By analysing the response models of several macroinvertebrates and fish trait-based metrics in a multiple-pressure context, the present study aimed at addressing several key questions: (1) Can fish and macroinvertebrates trait-based metrics detect the global pattern of marine ecosystem degradation? (2) Is it possible to distinguish single effects of

specific pressures in a multiple-pressure context? (3) Do both indicators detect specific pressures similarly (organic, fishing, physical, non-point-source)?

Material and Methods

Study area and human pressure gradients

The study area is located on the coastal shelf off Cascais and extends between Carcavelos (38º40'36''N 9º19'32''W) and Cabo da Roca (38º46'51''N 9º30'2''W), covering a depth range between 20 and 50 m and a marine area of 109 km^2 (Figure 6.1). The adjacent terrestrial area is highly populated (approximately 200.000 inhabitants) and consequently the study area is under the influence of multiple human pressures. These include a submarine sewage outfall (see Sampaio et al. 2010a for details), the influence of the Tejo estuary (see Vasconcelos et al. 2007 for details), bathing waters and polluted streams (see Viegas et al. 2009 for details), shellfish aquacultures in extensive regime, recreational (e.g. angling and spearfishing) and commercial (e.g. nets, pots, longlines) fishing activities, marina and anchoring areas, intensive recreational sport activities (sailing, windsurf, canoeing, surf, kitesurf, diving) and physical structures mainly related to urban and port development (Hidroprojecto 2008).

In order to understand how human-driven changes are distributed across pressure types, pressure sources were grouped into the following categories: organic pollution, fishing, physical and non-point-source (see Table 6.1 for details). In the present study, organic pollution only included the sewage outfall, since pressures that can result in several types of contamination were considered in the non-point-source category (high variety of pollutants).

Using a Geographical Information System (GIS) approach, "environmental risk surface" analysis was performed for each pressure type, with the purpose of classifying samples according to the level of influence of human pressures. This analysis consists of a modelled composite raster surface that combines information about the extent and relative intensities of perceived environmental risks in the studied area (Schill & Raber 2009). To do so, spatial information about each stressor was mapped into a layer, and a relative scale was used to rank each layer according to intensity (measure of the degree of risk), expected range of influence and weight (expected level of impact) (see Table 6.1 for details). Intensity varied between 1 and 5 and was obtained by ranking the classification data chosen for each pressure among locations (see Table 6.1 for details about data and metrics used for intensity classification). Mean values of classification data from the last 5

years were used whenever possible. Range of influence values were adapted from Ban and Alder (2008) and Ban et al. (2010) and complemented with inquiries to several local stakeholders and available legislation. A linear decay function was used to simulate decrease in pressure intensity with increasing distance to the pressure source. The weight values used resulted from the mean value of frequency of occurrence (1 - rare to 4 - persistent) and the expected degree of impact on the marine environment (1 - low to 4 - high). Weight values obtained were then normalized into a 1 to 3 scale (1 - low, 2 - medium and 3 - high) (see Table 6.1 for details). The assignment of frequency and expected impact values was performed according to the authors' judgement, based on the values indicated in Halpern et al. (2007).

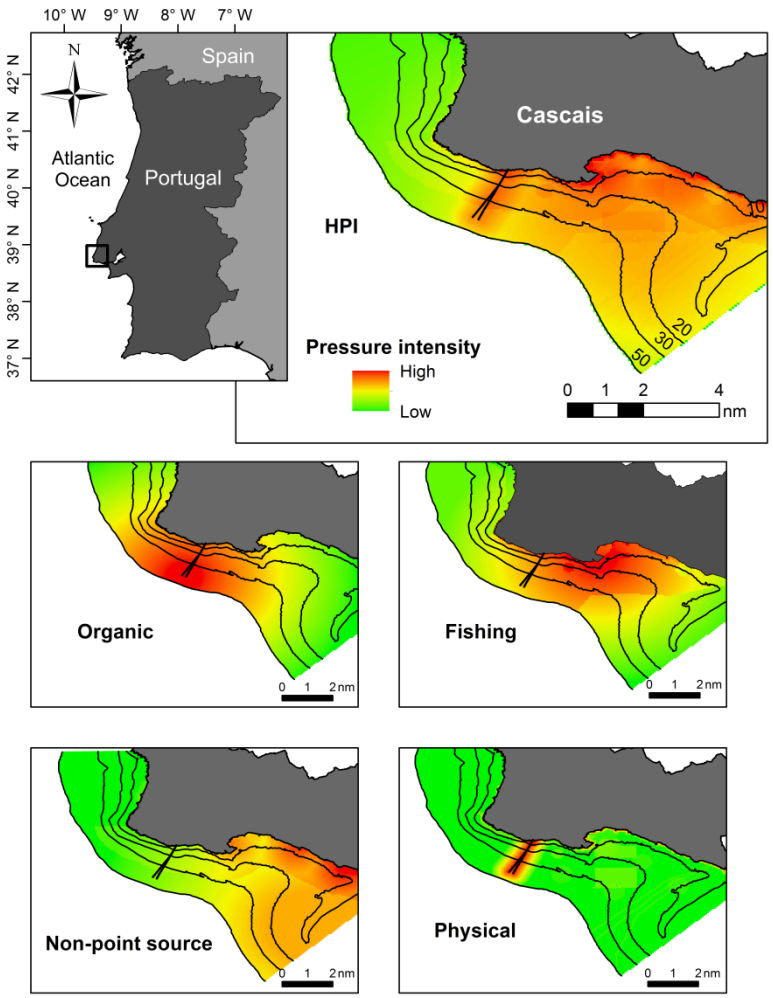


Figure 6.1 Map of the study area showing the spatial distribution of the four specific pressure types analysed, as well as the combined effect of those pressures in a Human Pressure Index (HPI).

Table 6.1 Relative scale used to estimate the four types of human pressure gradients (organic, fishing, physical, non-point-source), showing the ranks of each activity/pressure source according to their intensity (rank order from 1 - low to 5 - high), expected range of influence and weight according to the degree of expected impact (1 - low; 2 - medium; 3 - high).

Typology of pressure	Activities/ pressure sources	Metrics used to defined the intensity	Distance	Intensity	Weight
Organic	submarine sewage outfall	levels of *Escherichia coli* in water [a]*	10000 m	5	3
Fishing	fishing with angling from shoreline	number of emited licenses[a§]	100 m	3	2
	spearfishing	number of emited licenses[a§]	located	3	2
	fishing with angling from boat	number of emited licenses[a§]	9260 m from marina	5	2
	commercial fishing (e.g. nets, pots, longlines)	number of emited licenses[a§]	located[b]	4	3
Physical	physic submarine and shoreline structures	local knowledge	200 m	1-3[c]	2
	sailing competition areas	local knowledge	located	1	1
	anchoring areas	local knowledge	located	1	1
	recreational sport activities (sailing, windsurf, canoeing, surf, kitesurf)	local observations / stakeholders consultation	200 m	1	1
	diving	local observations / stakeholders consultation	located[b]	2	1
	use of beaches	number of concessions per beach area	200 m	1-3[c]	1
	marina (structure)	local knowledge	located	3	1
Non-point source	extensive shellfish aquacultures	local knowledge	located	1	2
	streams pollution	classification of Hidroprojecto 2008 report	2000 m	3	1
	Tejo estuary	based on Vasconcelos et al. 2007	10000 m	5	3
	bathing waters quality	levels of *Escherichia coli* in water[a]*	200 m	1-3[c]	2
	marina (water quality)	number of moorings in marina of Cascais[a]	200 m	3	2
	recreational boating	number of registered vessels[aβ]	9260m x 1852 m	5	1

[a] intensity values estimated by comparison with those values along the Portuguese coast

[b] mapped based on inquiries to professionals and legislation

[c] depends on its location

*source: National Institute of Water (INAG)

[§] source: Portuguese General-Directorate for Natural Resources (DGPA)

[β] source: National Institute of Statistic (INE)

For each type of pressure (organic, fishing, physical and non-point-source), a raster (100 m cell width) with the cumulative impact score (CIS) was created based on previous work by Halpern et al. (2008):

$$CIS = \sum_{i=1}^{n} A_i * w_i$$

where A_i is the intensity of each activity or human pressure source A in the location i, while w_i represents the weight given to each source for that location. Analyses were performed using the extension "Environmental Risk Assessment" of the package "Protected Area Tools v4" (Schill & Raber 2009) in ArcGIS 10 software. Ultimately, a Human Pressure Index (HPI) was created by combining (summing cell values) raster layers representing individual pressures, hence reflecting the cumulative impacts for each location.

Sampling strategy

During 2009, both fish and macroinvertebrates assemblages were surveyed in four sampling campaigns (March, June, September and November). In order to ensure that all the study area was equally covered in each sampling campaign, three sectors were delimited, where samples were randomly collected.

A total of 120 macroinvertebrate samples were collected using a 0.1 m^2 "Day" grab. These samples were then transported to the laboratory and washed over a 0.5 mm-mesh sieve. The material removed was conserved in ethanol (70%) and stained with Rose Bengal. Macroinvertebrates were sorted, counted and identified to the lowest taxonomic level possible (usually to genus/species level). The total density ($ind.m^{-2}$) per taxa was estimated for each replicate. Additionally, 100 g size of substrate were taken from each site in order to characterize the composition of bottom sediments (gravel - Ø > 2000 µm , sand - 2000 < Ø < 63 µm and mud- Ø < 63 µm) in percentage. All sample locations were recorded using a GPS (Global Positioning System) device.

Fish assemblages were sampled on board of a fishing vessel using an otter-trawl (12 m headline; 20 m footrope; 80 mm cod-end mesh), covering a total area of 280.452 m^2. A total of 24 hauls were performed, with a duration of 20 minutes each (6 in each sampling campaign). Hauls were carried out in daylight at a constant speed (2.1 - 2.3 knots) and all fish species were identified, measured (total length; 1 mm precision) and weighted (1 g precision). Geographic coordinates were recorded both at the start and end points of each

haul in order to estimate the total sampled area and calculate biomass (kg.1000m^{-2}) per species for each replicate.

Finally, values for organic, fishing, physical and non-point source pressure were attributed to each sample, by overlapping sample locations with the raster images containing the human pressure gradients.

Biological trait-based metrics

Based on an extensive revision of published literature on the expected response of trait-based metrics to human-induced pressures (Table 6.2), a total of 9 fish-based and 21 macroinvertebrate-based metrics were selected (Table 6.3). This was followed by the classification of all taxa of both indicators (fish and macroinvertebrates) according to their functional traits. Trophic levels, length at first maturity, mobility, trophic guild, commercial value and resilience traits of every fish species were assigned based on previous classifications by Henriques et al. (2008), updated with available literature and FishBase online database (Froese & Pauly 2012). As for macroinvertebrates, functional traits related with living habit, body size, environmental position and feeding habits were adapted from the classification by Bremner et al. (2003) and Aarnio et al. (2011) and classified according to information provided by the European Register of Marine Species (MarBEF 2013) and Biological Traits Information Catalogue (MarLIN 2006) online databases. The classification of ecological groups was done recurring to AMBI index software v.5 (AZTI´s Marine Biotic Index; Borja et al. 2000).

Data analysis

The response patterns of fish-based and macroinvertebrate-based metrics were analysed through distance-based linear models (DISTLM; Anderson et al. 2008). In order to assess if some of the metrics or a set of metrics were associated with specific pressure types and/or to a global pattern of cumulative pressures, a model was run for the following gradients: (1) univariate gradients (linear) - organic, fishing, physical and non-point-source pressures; (2) multivariate pattern - including all pressure types.

In order to identify the subset of trait-based metrics that best predicts the displacement of samples along each pressure gradient (coded into model matrices), metrics were subjected to a forward selection procedure and selected based on the corrected Akaike Information Criterion (AIC_c). AIC_c was used because it was developed to handle with

Table 6.2 Predicted changes in marine assemblages induced by anthropogenic pressures, as reported in several studies. Response trend: (+) increase (-) decrease (~) no change, with increasing levels of anthropogenic pressure.

Pressures	Biological element	Sensitive trait-based metrics	Response trend	Metrics explanation	Authors
Water contaminated by organic compounds	Fishes	Individuals with "low" and "very low" resilience	-	minimum population doubling time: more than 4.5 years	Russo 1982
		Rock residents	+	preference for rocky habitats (source of food and shelter)	Grigg 1994
		Omnivores	+	feed on detritus, alage, epifauna and infauna	Otway 1995
		Zooplanktivores	+	feed on planktonic crustacean, hydroids, fish aggs/larvae	Machias et al. 2005
		Detritivores	+	feed on detritus ans particulate organic matter	Tuya et al. 2006
		Invertebrate feeders	+	feed predominantly on non-planktonic invertebrates	Mckindley & Johnston 2010
		Tolerant-oportunistic individuals	+	benefit from their opportunistic life-history strategies or tolerance to the contamination	Henriques et al. submitted a
		Juveniles	+	bellow the maturity size	
	Macroinvertebrates	Proportion of pollution-sensitive species	-		Warwick et al. 1987
		Dominance of tolerant-opportunistic species	+	benefit from their opportunistic life-history strategies or tolerance to the contamination (e.g. Cirratulidae, Capitella spp., Nassarius reticulatus)	Borja et al. 2000
		Deposit-feeders	+	feeds on detritus that have settled on the bottom	Silva et al. 2004
		Scavengers	+	feeds on dead organic material (e.g. Nassarius reticulatus)	Cheung et al. 2008
		Filter/suspension fedeers	-	feed by straining suspended matter and food particles from water	Villnäs et al. 2011
		r-strategists	+	small rapidly breeding species	
		k-strategists	-	larger slowly growing species	
		Ecological group V (AMBI)	+	first-order opportunistic species (deposit-fedeers- close to anoxic)	
		Ecological group IV (AMBI)	+	first-order opportunistic species (small-size, short-life cycle)	
		Ecological group III (AMBI)	+	tolerant to excess organic matter	
		Ecological group II (AMBI)	~	indiferent to organic enrichment	
		Ecological group I (AMBI)	-	sensitive to organic matter- present in unpolluted conditions	
Water contaminated by a mixture of compounds (e.g. organic, chemical)	Fishes	Juveniles	-	bellow the maturity size	Khalaf & Kochzius 2002
		Herbivores	-	feed predominantly on macroalgae, macrophytes, phytoplankton and microphytobenthos	Mckindley & Johnston 2010
		Detritivores	+	feed on detritus	Henriques et al. 2013
		Zooplanktivores	+	feed on planktonic crustacean, hydroids, fish aggs/larvae	
		Invertebrate feeders	-	feed predominantly on non-planktonic invertebrates	
		Macrocarnivores	-	feed predominantly on macroinvertebra and vertebrates (mostly fish)	
		Omnivores	+/-	higher flexibility of diets, depends on the level of toxicity	
		Fish abundance	-	measure of abundance	
		Dominance of tolerant-opportunistic species	+	benefit from their tolerance to the contamination	

Table 6.2 (continued)

Pressures	Biological element	Sensitive trait-based metrics	Response trend	Metrics explanation	Authors
Water contaminated by a mixture of compounds (e.g. organic, chemical)	Macroinvertebrates	Deposit-feeders	+/-	subsurface- positive trend / surface- negative trend	Gaston et al. 1998
		Omnivores	+/-	higher flexibility of diets, depends on the level of toxicity	Borja et al. 2000
		Carnivores	-		Rakocinski et al. 2000
		Surface crawling	-		Oug et al. 2012
		Burrowers	+ / -	small burrowing nematodes ('+) / large burrowing bivalves (-)	
		Supension feeders	-	feed by straining suspended matter and food particles from water	
		Ecological group V (AMBI)	+	first-order opportunistic species (deposit-fedeers - close to anoxic)	
		Ecological group IV (AMBI)	+	first-order opportunistic species (small-size, short-life cycle)	
		Ecological group III (AMBI)	+	tolerant to excess organic matter	
		Ecological group II (AMBI)	~	indiferent to organic enrichment	
		Ecological group I (AMBI)	-	sensitive to organic matter- present in unpolluted conditions	
Fishing	Fishes	Macrocarnivores	-	feed predominantly on macroinvertebra and vertebrates (mostly fish)	Rochet & Trenkel 2003
		Invertebrate feeders	-	feed predominantly on non-planktonic invertebrates	Metharatta & Link 2006
		Individuals with high commercial value	-	directly related with the impacts of fishing which removes preferencially the large-bodied and target species	Auster & Link 2009
		Sedentary individuals	-	limited movement and well defined home ranges	Dimech et al. 2012
		Chondrichthyes	-	sharks, rays and chimeras	Henriques et al. submitted b
		Individuals with "very low" resilience	-	minimum population doubling time: more than 14 years	
		Dominance	+	number of species that make up 90% of the total biomass	
	Macroinvertebrates	Structure forming organisms	-	reef-forming organisms (e.g. *Sabellaria spinulosa*); increases habitat complexity	Thrush et al. 1998
		Large epifauna	-	large individuals that live on the surface of the substrate	Tillin et al. 2006
		Long-lived organisms	-	more than 5 years	Juan et al. 2007
		Dominance of opportunists	+	benefit from their opportunistic life-history strategies	Keiser & Hiddling 2007
		Dominance of scavengers	+	feeds on dead organic material	Dimech et al. 2012
		Burrowing individuals	-	lives and or moves in a tube	
		Filter/suspension feeders	-	feed by straining suspended matter and food particles from water	
		Short-livied organisms	+	less than 2 years	
		Deposit-feeders	+	feeds on detritus that have settled on the bottom	
		Sessil organisms	-	lives attached to the substrate	

situations where the number of samples (n) relative to predictor variables (q) is small (Anderson et al. 2008 and references therein). DISTLM analyses were performed on the basis of Euclidean distances between pairs of samples per pressure gradient and 9999 permutations were used to calculate significance (α = 0.05). Distance-based redundancy analyses (dbRDA) were used to visualize the response of each selected metric in the global pattern of cumulative pressures (i.e. best models for both indicators).

Table 6.3 Biological trait metrics used to describe the structural and functional response of the macroinvertebrates and fish to gradients of human pressure. See table 6.2 for metric descriptions.

Macroinvertebrates		**Soft-substrate fish**	
metric	code	metric	code
Density of very small individuals (<1cm)	Dvsmall	Biomass of invertebrate feeders	Binv
Density of small individuals (1-2cm)	Dsmall	Biomass of macrocarnivores	Bma
Density of small-medium individuals (3-10cm)	Dsma-med	Biomass of omnivores	Bom
Density of medium individuals (11-20cm)	Dmed	Biomass of sedentary individuals	Bse
Density of medium-large individuals (21-50cm)	Dmed-larg	Biomass of individuals with high commercial value	B€€€
Density of attached individuals	Dattached	Biomass of individuals with "low" resilience	BL
Density of tube-dweller individuals	Dtube	Dominance	DOM
Density of burrow dweller individuals	Dburrow	Biomass of Chondrichthyes	Bchon
Density of free living individuals	Dfree	Biomass of juveniles	Bjuv
Density of infauna	Dinfauna		
Density of epibenthic individuals	Depibenthic		
Density of deposit-feeders	Ddeposit		
Density of filter/suspention feeders	Dfilter		
Density of oportunistic/scavengers	Doportu		
Density of predators	Dpre		
Density of individuals of group I (AMBI)	group I		
Density of individuals of group IV (AMBI)	group IV		
Density of individuals of group V (AMBI)	group V		
Density of structure forming organisms	Dstruct		

In order to fulfill assumptions of linear modelling, Principal Coordinates Analysis (PCO) and draftsman plots were used to detect extreme multivariate outliers, visually evaluate when the slope of the relationship among metric values was notably skewed and identify redundant metrics using Pearson correlations ($|r| \geq 0.95$) (Clarke & Gorley 2006; Anderson et al. 2008). Fish-based metrics were fourth-root transformed, macroinvertebrate-based metrics were log(x+1)-transformed and all metrics and samples were kept, since no outliers and redundant metrics were identified (Clarke & Gorley 2006; Anderson et al. 2008). Although fish-based metrics related to rock residents and individuals with very low resilience were previously identified as sensitive to human pressure (Table 6.2), they were discarded from the present study due to their poor representativeness. Principal Coordinates Analyses (PCO) were performed based on Euclidean distances among all pairs of samples with all metrics previously normalized to place them on a comparable measurement scale.

All analyses were performed for fish and macroinvertebrates separately, but with all correspondent samples pooled together, using PRIMER 6 package with PERMANOVA+ (Clarke & Gorley 2006; Anderson et al. 2008). As macroinvertebrates assemblages are known to be strongly linked to sediment type (Thrush et al. 1998), the relationship between the log(x+1)-transformed macroinvertebrate-based metrics and sediment granulometry (gravel - Ø > 2000 µm, sand - 2000 < Ø < 63 µm and mud - Ø < 63 µm) was also tested through DISTLM analysis on the basis of Euclidean distances and 9999 permutations (α = 0.05).

Results

Although the study area is subject to multiple pressures acting on the same site, several differences were found in the expected spatial distribution of specific pressure types (Figure 6.1 and Table 6.1). In general, higher values of fishing and non-point-source intensities are expected near the shoreline. Despite that, the Tejo estuary seems to contribute greatly to non-point-source intensity values in areas closer to the river mouth (Figure 6.1). Physical pressure has a lower importance in most of the study area, with the exception of the small band around the submarine outfall structure. The sewage outfall is expected to affect much of the study area, with greater intensity near the mouth, located at a depth of 45 m (Figure 6.1).

None of the sediment type variables showed significant relationships with the macroinvertebrate-based metrics (DISTLM marginal tests: gravel Pseudo-F= 1.941 p>0.05, mud Pseudo-F= 2.098 p>0.05, sand Pseudo-F= 1.162 p>0.05), with the three sediment types together explaining only 3.3% of the total variation. This has led to the decision of discarding these variables from further analyses.

The best model results for macroinvertebrate and fish indicators suggest that they were both sensitive to the global pattern of cumulative pressures, as well as to some of the specific pressure types (Tables 6.4 and 6.5). The percentage of variation explained by the models of macroinvertebrate-based metrics ranged between 29.6% and 54.2%, with the exception of the physical pressure, where only 0.078% of variation was explained by the best model (AIC_c= 314.25) and thus it was excluded from results. In contrast, fish-based metrics explained higher percentages of variation (32.8%-73.4%). In both cases, a significantly higher number of metrics were selected for the non-point-source pressure models, which also explains the higher values of variation obtained for each biological indicator, whereas opposite patterns were observed for organic pollution.

Table 6.4 Distance-based Linear Model (DISTLM) analyses for macroinvertebrate-based metrics, showing both marginal and sequential tests performed with a forward selection procedure and AIC_c selection criteria. Marginal tests show how much variation is explained by each metric alone. Sequential tests explain the cumulative variation attributed to each metric, fitted in the model in the order presented. See table 6.3 for metric codes. Significant p-values in bold.

	MARGINAL TESTS				SEQUENTIAL TESTS						
	Metric	Pseudo-F	p-value	Proportion explained	Metrics selected	AICc	Pseudo-F	p-value	Proportion explained	Cumulative proportion	Response trend
Global	Dvsmall	2.479	**0.065**	0.021	group I	151.5	11.371	**0.000**	0.091	0.091	+
	Dsmall	8.858	**0.000**	0.073	Dmed	147.25	6.370	**0.001**	0.049	0.140	-
	Dsma-med	7.194	**0.000**	0.060	Dpre	143.11	6.234	**0.001**	0.046	0.186	+
	Dmed	1.514	0.196	0.013	Dstruct	141.29	3.904	**0.015**	0.028	0.214	+
	Dmed-larg	3.447	0.022	0.030	group IV	135.91	7.448	**0.000**	0.050	0.264	+
	Dattached	1.375	0.240	0.012	Dsmall	135.16	2.881	**0.044**	0.019	0.283	+
	Dtube	4.867	**0.004**	0.041	group III	134.73	2.573	0.054	0.017	0.300	+
	Dburrow	5.360	**0.002**	0.045							
	Dfree	6.044	**0.001**	0.051							
	Dinfauna	6.675	**0.001**	0.056							
	Depibenthic	8.919	**0.000**	0.073							
	Ddeposit	7.445	**0.000**	0.062							
	Dfilter	10.412	**0.000**	0.084							
	Doportu	5.932	**0.001**	0.050							
	Dpre	4.385	**0.009**	0.037							
	group I	11.371	**0.000**	0.091							
	group II	0.822	0.459	0.007							
	group III	4.940	**0.004**	0.042							
	group IV	5.283	**0.002**	0.045							
	group V	1.424	0.220	0.012							
	Dstruct	4.388	**0.009**	0.037							
Organic	Dvsmall	3.101	0.077	0.027	group I	244.62	22.083	**0.000**	0.163	0.163	+
	Dsmall	18.167	**0.000**	0.139	Dmed	229.98	17.557	**0.000**	0.113	0.277	-
	Dsma-med	12.187	**0.001**	0.097	Dfilter	229.09	2.970	0.088	0.019	0.296	+
	Dmed	0.661	0.419	0.006							
	Dmed-larg	4.220	**0.044**	0.036							
	Dattached	0.052	0.826	0.000							
	Dtube	5.296	**0.022**	0.045							
	Dburrow	9.231	**0.004**	0.076							
	Dfree	9.169	**0.003**	0.075							
	Dinfauna	12.279	**0.001**	0.098							
	Depibenthic	13.444	**0.001**	0.106							
	Ddeposit	11.337	**0.001**	0.091							
	Dfilter	17.681	**0.000**	0.135							
	Doportu	11.356	**0.001**	0.091							
	Dpre	5.514	**0.021**	0.047							
	group I	22.083	**0.000**	0.163							
	group II	0.363	0.539	0.003							
	group III	8.216	0.005	0.068							
	group IV	4.317	**0.040**	0.037							
	group V	1.439	0.236	0.013							
	Dstruct	2.868	0.094	0.025							

Table 6.4 (continued)

	MARGINAL TESTS				SEQUENTIAL TESTS						
	Metric	Pseudo-F	p-value	Proportion explained	Metrics selected	AICc	Pseudo-F	p-value	Proportion explained	Cumulative proportion	Response trend
Fishing	Dvsmall	4.768	**0.032**	0.040	Depibenthic	495.15	18.018	**0.000**	0.138	0.138	+
	Dsmall	7.846	**0.007**	0.065	Dstruct	487	10.442	**0.001**	0.074	0.211	+
	Dsma-med	11.678	**0.001**	0.094	group IV	474.64	14.925	**0.000**	0.094	0.305	+
	Dmed	0.434	0.507	0.004	Dmed	464.9	12.021	**0.001**	0.069	0.373	-
	Dmed-larg	7.378	**0.009**	0.061	Dattached	462.37	4.603	**0.034**	0.025	0.399	-
	Dattached	0.209	0.655	0.002							
	Dtube	12.008	**0.002**	0.096							
	Dburrow	1.756	0.190	0.015							
	Dfree	10.935	**0.001**	0.088							
	Dinfauna	8.233	**0.005**	0.068							
	Depibenthic	18.018	**0.000**	0.138							
	Ddeposit	7.745	**0.007**	0.064							
	Dfilter	8.171	**0.004**	0.067							
	Doportu	13.403	**0.000**	0.106							
	Dpre	12.467	**0.001**	0.099							
	group I	12.240	**0.001**	0.098							
	group II	1.059	0.303	0.009							
	group III	11.868	0.001	0.095							
	group IV	15.713	**0.000**	0.122							
	group V	3.786	0.053	0.032							
	Dstruct	14.471	**0.000**	0.114							
Non-point source	Dvsmall	1.882	0.171	0.016	Dfilter	651.76	15.876	**0.000**	0.123	0.123	-
	Dsmall	6.664	**0.010**	0.056	Dpre	644.52	9.486	**0.003**	0.068	0.192	+
	Dsma-med	5.719	**0.019**	0.048	Ddeposit	636.15	10.635	**0.002**	0.071	0.262	-
	Dmed	5.003	**0.028**	0.042	group IV	612.81	27.331	**0.000**	0.147	0.409	+
	Dmed-larg	0.256	0.619	0.002	Dstruct	605.87	9.054	**0.002**	0.045	0.454	+
	Dattached	5.316	**0.022**	0.045	Dinfauna	602.23	5.688	**0.017**	0.027	0.482	+
	Dtube	2.213	0.140	0.019	Dburrow	597.48	6.774	**0.010**	0.031	0.513	-
	Dburrow	10.608	**0.002**	0.086	Dsmall	595.56	4.018	**0.046**	0.018	0.530	+
	Dfree	4.219	**0.042**	0.036	group I	594.99	2.752	0.099	0.012	0.542	-
	Dinfauna	6.363	**0.014**	0.053							
	Depibenthic	4.708	**0.033**	0.040							
	Ddeposit	11.302	**0.001**	0.091							
	Dfilter	15.876	**0.000**	0.123							
	Doportu	0.240	0.626	0.002							
	Dpre	0.313	0.571	0.003							
	group I	11.641	**0.000**	0.093							
	group II	1.840	0.179	0.016							
	group III	0.409	0.519	0.004							
	group IV	1.891	0.172	0.016							
	group V	0.544	0.466	0.005							
	Dstruct	0.980	0.338	0.009							

Table 6.5 Distance-based Linear Model (DISTLM) analyses for fish-based metrics, showing both marginal and sequential tests performed with a forward selection procedure and AIC_c selection criteria. Marginal tests show how much variation is explained by each metric alone. Sequential tests explain the cumulative variation attributed to each metric, fitted in the model, in the order presented. See table 6.3 for metric codes. Significant p-values in bold.

	MARGINAL TESTS				SEQUENTIAL TESTS						
	Metric	Pseudo-F	p-value	Proportion explained	Metrics selected	AICc	Pseudo-F	p-value	Proportion explained	Cumulative proportion	Response trend
Global	Binv	4.565	**0.006**	0.172	**Binv**	32.30	4.565	0.005	0.172	0.172	+
	Bom	2.560	0.060	0.104	**Bma**	30.12	4.650	0.006	0.150	0.322	-
	Bma	3.367	**0.025**	0.133	**Bom**	29.45	3.214	0.025	0.094	0.416	+
	Bse	2.389	0.074	0.098							
	B€€€	0.342	0.807	0.015							
	BL	2.730	0.051	0.110							
	DOM	0.904	0.440	0.039							
	Bchon	1.349	0.263	0.058							
	Bjuv	1.559	0.203	0.066							
Organic	Binv	10.744	**0.003**	0.328	**Binv**	39.08	10.744	**0.005**	0.328	0.328	+
	Bom	0.863	0.355	0.038							
	Bma	0.091	0.761	0.004							
	Bse	1.926	0.182	0.080							
	B€€€	0.389	0.536	0.017							
	BL	0.144	0.720	0.007							
	DOM	0.492	0.487	0.022							
	Bchon	0.113	0.743	0.005							
	Bjuv	0.475	0.498	0.021							
Physical	Binv	2.466	0.132	0.101	**Bjuv**	-51.95	3.358	0.081	0.132	0.132	-
	Bom	1.199	0.276	0.052	**Bom**	-53.93	4.447	**0.049**	0.152	0.284	+
	Bma	2.217	0.153	0.092	**Bma**	-54.38	2.998	0.096	0.093	0.377	-
	Bse	0.327	0.629	0.015							
	B€€€	0.203	0.662	0.009							
	BL	2.876	0.094	0.116							
	DOM	2.400	0.154	0.098							
	Bchon	0.000	0.993	0.000							
	Bjuv	3.358	0.080	0.132							
Non-point source	Binv	0.226	0.644	0.010	**Bma**	130.98	12.481	**0.002**	0.362	0.362	-
	Bom	5.074	**0.035**	0.187	**B€€€**	123.89	10.492	**0.004**	0.213	0.575	+
	Bma	12.481	**0.002**	0.362	**Bse**	119.47	7.139	**0.017**	0.112	0.686	+
	Bse	9.231	**0.006**	0.296	**Bjuv**	118.69	3.453	0.075	0.048	0.735	-
	B€€€	0.776	0.386	0.034							
	BL	6.899	**0.017**	0.239							
	DOM	0.841	0.347	0.037							
	Bchon	4.992	**0.033**	0.185							
	Bjuv	2.691	0.115	0.109							
Fishing	Binv	7.267	**0.015**	0.248	**Binv**	101.90	7.267	**0.013**	0.248	0.248	+
	Bom	3.583	0.070	0.140	**BL**	97.23	7.458	**0.013**	0.197	0.445	-
	Bma	1.741	0.202	0.073	**Bom**	96.81	2.978	0.103	0.072	0.517	+
	Bse	0.025	0.876	0.001	**Bse**	95.16	4.274	0.050	0.089	0.606	+
	B€€€	0.016	0.902	0.001							
	BL	1.934	0.178	0.081							
	DOM	0.017	0.897	0.001							
	Bchon	0.940	0.349	0.041							
	Bjuv	0.048	0.833	0.002							

The dbRDA plots in figures 6.2 and 6.3 represent the best combination of metrics that responded to the global pattern of cumulative pressures for macroinvertebrates and fish indicators, respectively.

Regarding macroinvertebrates assemblages (Table 6.4; Figure 6.2), the analyses of metric response trends along pressure gradients indicate that the organic and non-point-source impacts were probably the most responsible for the global cumulative pattern. In this case, the first two metrics selected in best model for the global pattern (ecological group I and medium-sized individuals) varied in the same direction of organic pollution, while the response of the remaining metrics was consistent with the non-point-source models (predators, ecological groups III and IV, small and structure forming individuals).

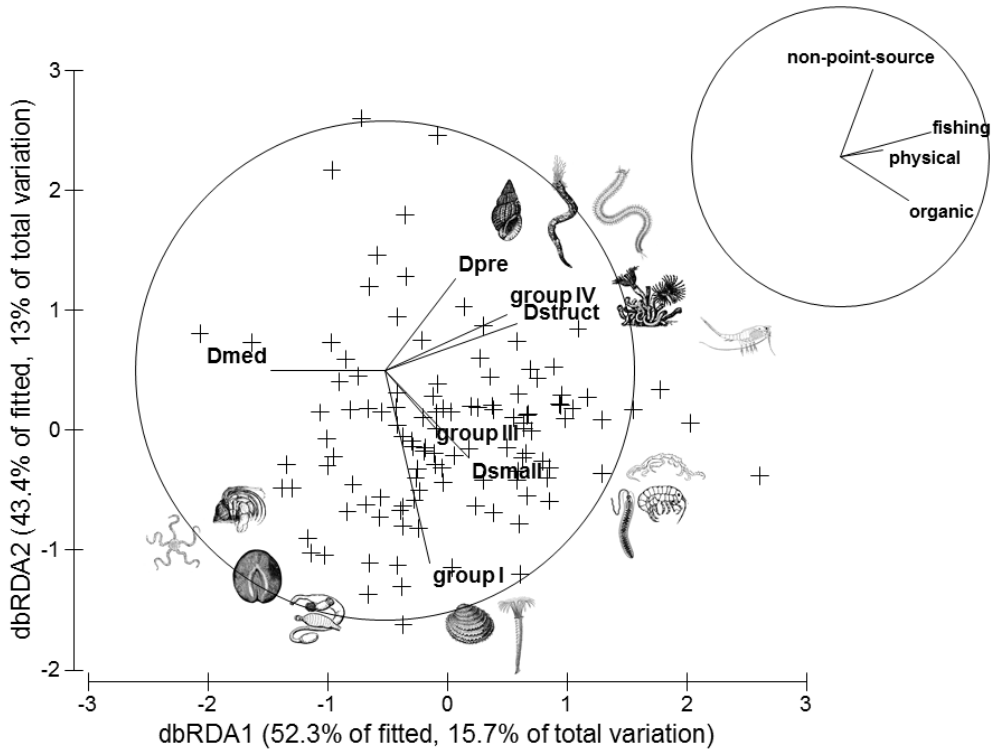


Figure 6.2 Distance-based Redundancy Analysis (dbRDA) showing best model results for macroinvertebrate-based metrics and their correlations with the axes (circles represent vector correlations of 1). Ordination plot with position of pressure gradients is shown on the top. See table 6.3 for metric codes. Some macroinvertebrate taxa are also illustrated and images are from Clipart courtesy FCIT (http://etc.usf.edu/clipart/).

Moreover, filter/suspension feeders and ecological group I were selected for the best models of these specific pressures, but showed opposite trends (i.e. increased values in response to organic pollution and decreased values with the increased intensity of the non-point-source pressure). Metrics related to epibenthic and attached individuals were only selected in the best model for fishing impacts. However, contrary to the expected, epibenthic and structure forming individuals were positively associated with this pressure. Ecological group IV and I, filter/suspension feeders and structure forming individuals were the most selected metrics overall.

When compared to macroinvertebrates, fish-based metrics reflected that not only organic and non-point-source pressures contribute greatly to the global pressure pattern, but also that fishing and physical pressures could influence the global pressure patterns (Table 6.5; Figure 6.3).

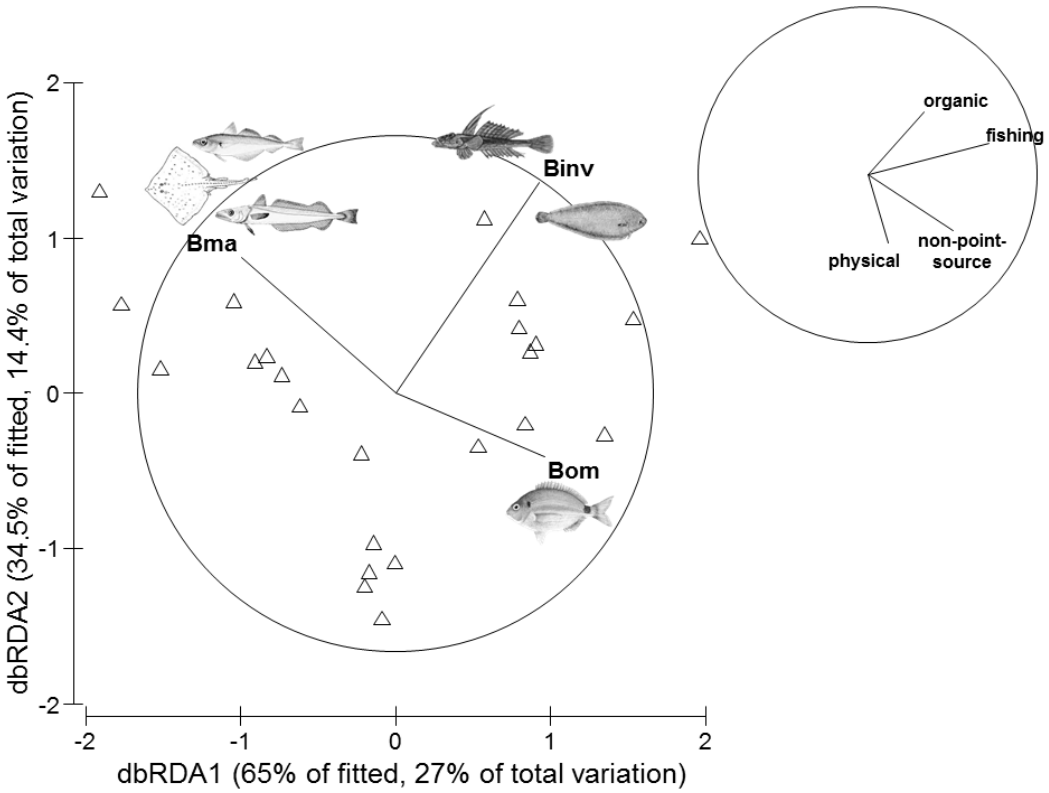


Figure 6.3 Distance-based Redundancy Analysis (dbRDA) showing best model results for fish-based metrics and their correlations with dbRDA axes (circles represent vector correlations of 1). Ordination plot with position of pressure gradients is shown on the top. See table 6.3 for metric codes. Some representative fish species are also illustrated. Fish images were adapted from Food and Agriculture Organization of the United Nations (FAO).

However, only metrics related with trophic structure responded significantly to the global pattern of cumulative pressures. Invertebrate feeders explained 17% of variation in the global pressures model, changing in the same direction of organic and fishing pressures, whilst macrocarnivores decreased with the increased values of non-point-source intensity and explained 15% of variation in the global pattern of cumulative pressures (Table 6.5). Omnivores only explained 9% of variation in the global model and their trend seems to be linked to physical changes (significant results and higher proportion of explained variation), despite that, this metric had individual significant differences in marginal tests within non-point-source pressures. Besides macrocarnivores, sedentary and high commercial value individuals have shown a significant positive trend in the face of non-point-source pressure. Although not significant, juveniles showed a consistently negative trend in response to non-point-source pressure and individuals with low resilience decreased significantly with the increase of fishing pressure.

Discussion

This study highlighted the usefulness of trait-based metrics in the assessment of human-induced changes in coastal areas, since both biological indicators detected the global pattern of cumulative pressures. However, given the expected response of the tested metrics (see Table 6.2) and the results obtained in the models, it seems that the effects of the global pattern of cumulative pressures were mainly due to non-point-source pressures and not to a combined effect of all pressures, even though some variability was explained by organic pollution. This possibly means that the detection of pressure-specific effects will depend in part on the magnitude, persistency and spatial scale of disturbances, emphasizing the difficulties of assessing single pressure effects in a multiple pressures context on a broad range ecosystem.

In many cases, trait-based metrics of both indicators responded predominantly to specific pressures, although this specificity was obscured by different pressures acting on the same area. As evidenced in freshwater ecosystems (e.g. Hering et al. 2006; Johnson et al. 2006; Marzin et al. 2012), it is possible that losses caused by a specific pressure could be compensated by some benefits provided by other pressure (e.g. toxicity vs. food supply), which makes the biological assessment in a multiple pressures context much more complex, with likely confounding effects. These facts might explain why some metrics were unexpectedly selected for pressure-specific models in both macroinvertebrates and fish indicators, suggesting that, in a multiple pressure approach, the analysis of indicator

response should focus on known sensitive metrics, giving attention to the remaining metrics only if they put forward contradictory results.

Non-point-source pollution can result in a mixture of contaminants with different degrees of toxicity (Islam & Tanaka 2004; McKinley & Johnston 2010), thus, it was somewhat expected that a higher number of metrics from both indicators were sensitive to this source of pollution. In fact, several studies have highlighted strong relationships between pollution (e.g. run-off and/or industrial wastewaters) and decreases in diversity, abundance (e.g. Giangrande et al. 2005; Johnston & Roberts 2009; McKinley & Johnston 2010), as well as with changes in some biological traits and ecological groups (Gaston et al. 1998; Borja et al. 2000; Khalaf & Kochzius 2002; Oug et al. 2012; Henriques et al. 2013). Besides several small polluted streams (Viegas et al. 2009), marina wastewaters and other non-point sources of pollution, the studied area is affected by the Tejo estuary, which is not only a source of domestic and industrial wastewaters, but also an important cause of physical disturbance, as the movement of sediments can create unstable conditions on the bottom (see Silva et al. 2004).

In macroinvertebrate assemblages, the response of ecological groups (AMBI), small-sized and filter/suspension feeders to pollution is supported by a well-established and accepted scientific knowledge (e.g. Gaston et al. 1998; Borja et al. 2000; Borja et al. 2009; Azevedo et al. 2011). In this context, the results obtained for ecological groups I (decreasing trend of species sensitive to organic matter) and IV (increasing trend of opportunistic species), small individuals (increasing trend) and filter/suspension feeders (decreasing trend), suggests the presence of some degree of pollution stress in the areas most influenced by non-point-source pressures. In accordance, there were some evidences that macrocarnivores and juveniles of fish assemblages responded accurately to this source of pollution (negative trends) (Khalaf & Kochzius 2002; Henriques et al. 2013). The higher densities of predators, infauna (macroinvertebrates) and omnivores (fish) could be associated with the unstable conditions of the bottom, as they profit from higher mobility and/or flexibility of diets (Khalaf & Kochzius 2002; Oug et al. 2012).

Taking into account the results previously obtained at this sewage outfall, which showed changes in both macroinvertebrates and fish assemblages (Silva et al. 2004; Santos et al. 2008; Sampaio et al. 2010b; Sampaio et al. 2011; Henriques et al. submitted-a), a weak response of metrics to organic pollution was found in the models of both indicators. Although one might think at first that estuarine outflow dominated the study area, masking the effects of sewage discharges, the most probable explanation is the lack of samples in the area nearest the sewer mouth. In fact, those studies only found significantly higher

values in opportunistic and scavenger macroinvertebrates (e.g. *Capitella* spp. *Nassarius reticulatus*), as well as in fish guilds (invertebrate feeders and rocky resident fish) at the area closest to diffusers (~4 km), which was attributed to the hydrodynamic stress that promotes the early dilution of wastewaters (Santos et al. 2002). Moreover, Sampaio et al. (2010b) noticed higher influence of sewage dispersion up to 500 m from the outfall through the use of carbon and nitrogen isotopes to trace sewage-derived organic matter in macroinvertebrates and sediments, whereas Santos et al. (2008) detected high concentrations of fecal coliforms up to 2 km. Despite that, invertebrate feeders (fish) showed a significant response to the organic gradient, and the macroinvertebrates ecological group III (tolerant to organic matter) and small-sized individuals seem to be associated with organic pressure within the global pattern of cumulative pressures, according to the expected trends (see Table 6.2).

All these facts indicate that probably more accurate results are obtained when the sampling design is directed to pressure sources (e.g. sampling along a pre-defined pressure gradient instead of randomly). If sampling is performed on randomly placed locations (like the present study), it is possible that indicators fail to detect pressures or show weaker responses, responding preferentially to pressures with higher spatial extension (e.g. non-point-source). This directed sampling approach could have strong implications in the success of local management plans.

In contrast with the two mentioned pressure types, where some predictable metrics responded to increases in intensity, fishing and physical pressure gradients revealed unexpected responses (e.g. macroinvertebrates - epibenthic and structure forming individuals; fish - sedentary individuals and invertebrate feeders). Moreover, many of the most sensitive metrics to fishing pressure were not selected (e.g. high commercial fish and chondrichthyes) (see Table 6.2 for details). While no strong impact was expected from physical structures, fishing was expected to have some detectable impacts. These results may suggest that fishing impact is not high enough in the study area to be detected or, at least, the fishing gears used may not be destructive enough to cause strong changes in both fish and macroinvertebrate assemblages. Actually, to our knowledge, the majority of the studies that analysed functional and structural changes on soft-substrate marine assemblages focus on trawling pressure (Thrush et al. 1998; Tillin et al. 2006; Kaiser & Hiddink 2007; Auster & Link 2009; Dimech et al. 2012; Henriques et al. submitted-b). Since most of the local fishermen use pots and nets, the lack of detection could be attributed to weak sensitivity of the metrics tested, maladjustment of the spatial area defined as impacted by fishing, or to actual low impact of fishing activities when compared with other

pressures. Nonetheless, further research should be conducted to relate local fishing effort (e.g. traps, pots, nets, longlines) with changes in the structure and function of invertebrates and fish assemblages.

Overall, the present study clearly supports the use of structural and functional trait-based metrics in the design of monitoring plans, as both indicators similarly detected the most impacted sampled areas and mutually support evidence of weak responses to some other pressure types (organic matter loads and fishing), independently of the causes of those responses. Although different sensitivities to perturbation would be expected between the tested indicators, none seemed to perform clearly better in the assessment of any of the specific pressures. Previous studies provided some proof that macroinvertebrates react to lower levels of perturbation due to their limited mobility and high dependence on the substrate, being apparently more affected by contaminants (Hering et al. 2006; Marzin et al. 2012). On the other hand, fish respond more dramatically to strong perturbations (including at large scales) from the moment when habitat conditions are no longer favourable, being probable early-warning indicators of recovery (Marzin et al. 2012). In this way, information provided by the use of multiple indicators might be complementary and give a more complete picture when assessing global degradation patterns. However, further research about indicator sensitivity to different types and intensities of pressure is urgent, in order to select potentially robust early-warning indicators/metrics to assess the quality of marine ecosystems.

In a broad range ecosystem, without defined boundaries between habitats, an approach similar to the one applied, i.e. previously identifying the expected pressure sources and then analysing if the biological indicators detect changes, together with directional monitoring plans (pressure-sources vs. controls) could be the only way to accurately assess human impacts on marine ecosystems, while making the local/national management plans more cost effective. Additionally, as structural and functional metrics simplify taxonomic data into information that is more understandable for the general public (Azevedo et al. 2011; Henriques et al. 2013) and as they are well-adapted for broad-range geographical scales (Henriques et al. 2013), their use could also have significant implications, not only in the success of local management plans, but also to fulfil the requirements of international policies, such as the Marine Strategy Framework Directive.

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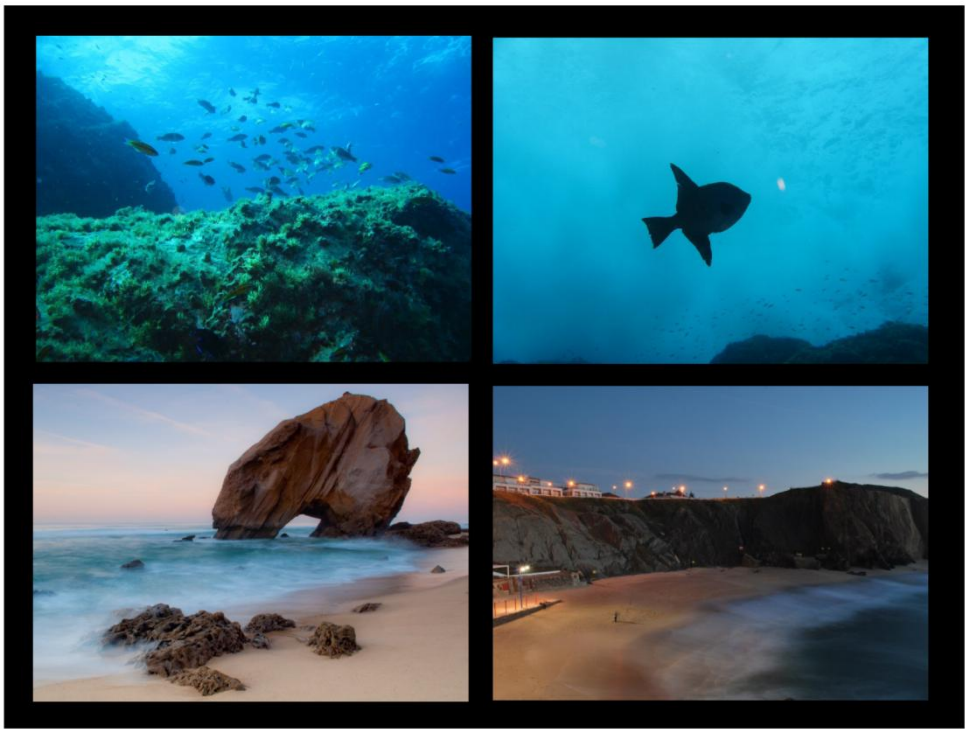
CHAPTER 7



Conclusions and final remarks

Conclusions and final remarks

Overall, this thesis has clearly demonstrated the usefulness of metric-based approach for characterizing changes in fish assemblages due to anthropogenic pressures and represents an important contribution towards a deeper understanding of the consequences of those pressures. Moreover, to our knowledge, this thesis comprises the first integrative metric-based approach to assess changes in marine fish assemblages and identifies some sets of metrics sensitive to the main anthropogenic pressures acting on marine ecosystems, such as fishing and different water contamination sources.

The assessments of fish assemblages associated with both rocky (Chapter 2) and soft-substrate habitats (Chapter 4 and 5) showed that the metric-based approach provides stronger evidence of anthropogenic-induced changes than species individually. Besides providing more understandable information (Elliott et al. 2007; Mouillot et al. 2012), in some cases the observed changes in the abundance or biomass of particular species were balanced by changes in other species that shared the same biological trait, hence not altering some features of the assessed assemblages (compensation mechanisms) (Chapter 2, 3, 4 and 5). For instance, no structural and functional shifts were found in the response of rocky fish assemblages to a thermal effluent, despite the observed differences in species composition between disturbed and control sites (Chapter 2). This mechanism of compensation, due to functional redundancy, has been largely recognized as one of the most important factors responsible for the resilience and stability of ecosystems, making the use of a metric-based approach more powerful in the assessment of ecological condition of ecosystems (Hughes et al. 2005; Bremner 2008; Mouillot et al. 2012). On the other hand, when pressure is severe enough to affect almost all the species representing a given structural or functional trait, this compensation mechanism becomes less efficient, with consequent loss of that stability (Hughes et al. 2005; Bremner 2008; Mouillot et al. 2012). Therefore, it is not surprising that different patterns of response may be observed depending not only on the intensity and degree of destructiveness of pressure, but also on the complexity of habitat and biotic interactions affected.

Considering the assumption of limited resources, ranking the vulnerability of marine ecosystems and the impacts of anthropogenic pressures becomes an important task in

order to prioritize conservation efforts (Halpern et al. 2007; Crain et al. 2009; Ban et al. 2010). Since the analysed pressures had different potential degrees of destructiveness, i.e. trawling (soft-substrate) versus artisanal fishing activities (rocky reefs) and organic effluent (soft-substrate) versus mixture of compounds from sewage discharges and run-off sources (rocky reefs), it is not possible here to directly compare the susceptibility of fish assemblages of different habitat typologies. Rocky reefs, hard-bottom shelf areas, coral reefs and mangroves have been pointed out by experts as the most threatened marine ecosystems (Halpern et al. 2007). However, the selection of protected/recovery areas is ultimately associated with the local conservation targets and human uses.

Fish assemblages of rocky reef habitats were broadly affected by water pollution resultant from the mixture of potentially toxic components (sewage discharges and non-point sources of pollution) which led to changes of many metrics representing several attributes (trophic, mobility, structure, resilience, habitat, nursery function) (broad-range pressure), whereas fishing affected fish assemblages differentially, with specific metrics responding to its presence (selective pressure) (Chapter 2 and 3). In agreement, sewage discharges led to broad range effects on several attributes of fish assemblages while trawling led to more selective effects in the associated fish assemblages (Chapter 4 and 5). Although at first the number of sensitive metrics could be seen as a reflection of the degree of destructiveness caused by pressure, this is not necessary true since only one biological indicator and one specific pressure were evaluated. Thus, it is possible that broad-range pressures may affect more attributes of a given assemblage but cause less impact than a more selective pressure. For instance, demersal destructive fishing (e.g. trawling) has been widely recognized as one of the worse threats of marine ecosystems (Halpern et al. 2007; Crain et al. 2009; Dimech et al. 2012).

Present results, stress the urgent need for the development of integrative strategies to assess biologically and ecologically sensitive areas, rank human activities and their impacts, as well as analyse the spatial scale in which a given pressure disturbs, in order to design appropriate monitoring and management plans to achieving the sustainable use of the seas, e.g. Ecosystem-based management and Marine Spatial Planning (MSP) (Crowder & Norse 2008; Douvere 2008; Levin et al. 2009; Foley et al. 2010). Yet, these approaches raise a question: how to assess anthropogenic pressure effects in changing ecosystems? Although marine ecosystems are subject to multiple pressures and are spatially and temporally dynamic (Costanza & Mageau 1999; Cury et al. 2003; Rice 2005; Johnson et al. 2012), which makes their assessment difficult, several aspects of the applied sampling method may contribute greatly for the success of monitoring plans, while

making them more cost-effective, as evidenced by several of the present findings, as follows:

(1) Given the influence of the environmental features in the composition of fish assemblages, such as habitat complexity, depth, biotic cover, sediment type, seasonal and inter-annual variability, among others (e.g. Holbrook et al. 1994; García-Charton & Pérez-Ruzafa 2001; Magill & Sayer 2002; Friedlander et al. 2003; Labropoulou & Papaconstantinou 2004; Henriques et al. 2007; La Mesa et al. 2011a), and the agreement between the observed response with the results previously obtained by other authors that analysed similar pressures at the species level (see chapters 2, 3, 4 and 5 and references therein), the general applied approach in this thesis seems to be adequate to detect anthropogenic impacts. Accordingly, future studies aimed at assessing changes in fish assemblages should follow the same commencement, i.e. minimize the effects of natural variability by comparing habitats of similar complexity and accounting for season and/or inter-annual shifts. In this way, the use of measures of habitat complexity (Pais et al. 2013), substrate cover, biotic cover, exposure and depth, is advisable in order to characterize rocky reef habitats (Chapter 2 and 3). Soft-substrate habitats should be characterized at least using measures related with depth, sediment type, exposure and latitude (Chapter 4 and 5);

(2) Despite the general idea that structural and functional guilds are more resilient to natural variations than species abundances (Elliott et al. 2007; Henriques et al. 2008), results of chapter 3 showed that seasonal variations can influence the patterns of some fish-based metrics and in turn potentially affect the detection of changes in rocky reefs depending on the intensity and degree of damage caused by pressure. In this case, the choice of a specific season to survey rocky reefs, during the warm season after the spawning period (July-October), seems to be more adequate to detect changes in fish assemblages, while minimizing monitoring costs (Chapter 3). In this context, further research is needed to suitably assess the effects of seasonal variations in soft-substrate fish-based metrics and uncover the best season to detect anthropogenic impacts;

(3) A novel approach to select sensitive metrics was tested in chapter 5, by modelling the response of several fish-based metrics to a gradient of trawling intensity and comparing the consistency of those metrics among four different habitat typologies. This approach proved to be efficient in the assessment of extensive marine areas as it allowed the detection of sensitive metrics against a background of natural variability. Consequently, it

can have deep implications in the assessment of anthropogenic pressures that embrace broad areas, and therefore, it should be tested for other types of pressure sources;

(4) Chapter 6 highlighted some findings that are of extreme importance to deal with multiple pressures contexts and broad scales, since they show the weaknesses of common monitoring plans, which are designed to characterize marine assemblages and may not be able to detect specific anthropogenic pressure effects. Overall, the results reinforce the difficulties of detecting single pressure effects, which is of paramount importance for increase management options, due to the lack of pressure-specific metrics and the influence of multiple pressures that act synergistically upon the assemblages (both fish and macroinvertebrates indicators). In this way, an alternative approach is recommended to improve pressure specific analysis, which consist firstly of identifying the expected pressure sources and applying a directional monitoring plan (pressure vs. controls), to analyse if the biological indicators detect changes (chapter 6). On the other hand, this directed monitoring plan probably makes the local/national management plans more cost-effective.

The advantages of using metric-based approach are evident, given the diversity of natural factors that can influence the detection of anthropogenic pressures. Although the selected fish-based metrics seem promising in the assessment of anthropogenic pressures, the present study is but a starting point for the successful use of marine fish assemblages as indicators. The development of this approach would highly benefit from further research including spatial (higher number of rocky reefs and soft-substrate habitats and other biogeographic regions) and temporal (seasonal and inter-annual) variability in the response of fish-based metrics. Moreover, these investigations should also address not only the assessed pressures, but also other drivers of pressure such as dredging activities, aquacultures, other fishing activities, other types of water pollution. Such studies should allow to increase base-knowledge about fish assemblages changes, find potential new sensitive metrics, test the broad applicability of fish-based metrics and reinforce metrics sensitiveness.

Most assessment tools (multimetric indices) developed for fish assemblages in several aquatic ecosystems compare the observed metric values with a reference scale, based on the values that the metrics would have in the absence of anthropogenic pressure, to classify the final ecological condition (e.g. Karr 1981; Deegan et al. 1997; Harrison & Whitfield 2006; Hering et al. 2006; Roset et al. 2007). Even though some compensatory mechanisms reduce the influence of natural variability and given the amount of factors that

determine the homogeneity of marine assemblages spatially and temporally, here the application of a common reference method would probably lead to inadequate classifications. Therefore, an alternative classification method on a case-by-case basis based on the percentage of deviance between control and disturbed sites, by pressure, would probably assess more accurately the ecological conditions with higher degree of confidence. To test this hypothesis, a strong background of the results of above-mentioned studies is needed to define those percentages of deviance. Until then, another option may be the simple assessment of has/has no impact or analysing gradients of pressure as in the present study.

Finally, to achieve an efficient sustainable use of marine resources, the pressure sources must be identified to assure the establishment of efficient management plans. However, it is difficult to find in practice metrics sensitive to single pressure (pressure-specific) due to its scattering power over the complex networks of biotic/abiotic interactions (Niemi et al. 2004). In this way, the proposed directed monitoring approach (chapter 6), which follow the principles of the ecosystem-based Marine Spatial Planning (MSP) (Crowder & Norse 2008; Douvere 2008; Levin et al. 2009; Foley et al. 2010), should be tested and include other biological indicators (e.g. algae, invertebrates, phytoplankton), in order to increase the knowledge about the effects of anthropogenic pressures on marine assemblages and properly assess the ecological condition of marine ecosystems.

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